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STUDIES ON BACILLUS ANTHRACIS

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STUDIES ON BACILLUS ANTHRACIS

INTRODUCTION

Woolsorters' Disease

"Now as in the case of other specific poisons, so in this one of anthrax, it is not all classes of animals that are equally susceptible to its influence; nor is the same animal at all times and under all circumstances capable of being infected. That the human race is one showing an inferior susceptibility to the infection is a conclusion I think fully justified. Doubtless many cases of anthrax in man occur, and pass unrecognised (the experience of Bradford shows it), and the certainty that it is so is a point worthy the attentive consideration of the medical profession generally; but having regard to the manifold ways in which the poison must be distributed over the country -- ways numerous it seems beyond all previous conception even from this one manufacture, we can only explain the unrecorded occurrence of the disease amongst the general population by acknowledging this inferior susceptibility. If, too, it were readily transmissible to man, we should, I certainly think, having regard to the nature of the occupation, expect the woolsorters themselves to suffer more frequently; and, when they become infected, we might expect the disease to spread sometimes to others; for experiments on animals prove that the poison as it exists in the human body is actively infective. The behavior of the disease itself, again, a study of what has preceded in the text of the Report will show, greatly favours this conclusion. There is considerable evidence, for example, that the morbid material may remain for a variable time latent in the body. In man it is at any rate much less sudden in its effects than in the more susceptible species of animals. I have no cases of so-called "lightning" or "apeplectiform" attack to record. The disease in its stage of invasion differs widely too, not only from these cases amongst animals, but in different individuals. Thus this stage of invasion or prodromata is apparently of indeterminate course, it may sometimes be prolonged; the symptoms may even apparently be intermittent. The evidence, too, is almost conclusive that the disease often passes little or not at all beyond this stage; that, in fact, it aborts. Further, since the bacillus anthracis is rarely if ever found swarming in the blood of any animal until death is at least imminent, a superior power of resistance to the progress of the disease, such as the conditions above remarked upon indicate, is, we might conclude, dependent, not upon any exceptional individual tolerance of the presence of the contagion, but upon some actual restraining influence affecting the intrinsic power of development, and of diffusion, of the contagion itself. The whole picture of the infective process in many of these cases of anthrax in man is suggestive, then, in a peculiar degree, of a poison, at first and for a variable time, to use an expression of Darwin's 'barely able to prolong its existence'; but with the breaking down of unknown barriers, or with the advent to the blood or tissues of something favourable to its rapid multiplication, or perhaps in both these contingencies, there quickly and often most unexpectedly ensue the characteristic tumultuous course of the fully developed disease and swift and sudden termination.

"Will our knowledge allow to suggest an explanation of these peculiarly marked phenomena of individual susceptibility or immunity in anthrax? Their explanation is necessary to a due understanding of the pathology of the disease, or for its scientific treatment; and every circumstance that appears to bear upon the subject is worthy therefore of discussion."

From "On the so-called 'Woolsorters Disease' as observed at Bradford and in Neighboring Districts in the West Riding of Yorkshire", by Mr. John Spear, Rep Med Off Local Govt Bd 1830, App. A, No. 8, pp. 131-132, 1881.

STUDIES ON BACILLUS ANTHRACIS

PART 1

RESPIRATORY ANTHRAX IN DOGS, PIGS, AND SHEEP (Gochenour, Gleiser, Ward, Overholt, Huff and Tigertt)

I. INTRODUCTION

This study on Bacillus anthracis was undertaken to provide familiarization with the disease in large animals and information on the pathogenesis of respiratory anthrax in dogs, pigs, and sheep.

Specific objectives were determination of: (1) sites of primary host-parasite interaction, (2) defense mechanisms involved, (3) manner and routes of spread from areas of primary involvement, and (4) early recognition of infection. The early diagnostic approaches considered were demonstration of bacteremia or specific toxemia and enlargement of such thoracic lymph nodes as might be demonstrable by roentgen examination.

II. MATERIALS, METHODS, AND RESULTS

A. EXPOSURE DEVICE

The information sought in these studies required observation and serial sacrifice of large animals simultaneously exposed to a common respiratory challenge of B. anthracis. No suitable exposure device capable of such exposure conditions was available. It was therefore necessary to design, fabricate and test a device for this specific study. The limitation imposed by safety, as well as the requirement for supporting laboratory and animal holding space, dictated the location at Dugway Proving Ground (DPG). A report is available on the details^{1/}.

A simple, rectangular wind tunnel, approximately 100 ft long and 6 ft in cross-section was used (Figure 1). At the upwind end a standard fixture (Figure 2) was used to generate the aerosols. Animal exposure ports and impingers (Figure 3) were located on either side. Deep-bed bacterial filters (Figure 4) were located further downwind.

Animals to be exposed were placed on litters (Figure 5) exterior to the tunnel; their heads were inserted through rubber diaphragms opening to the interior (Figure 6).

A series of calibration tests using Bacillus subtilis var. niger as a simulant demonstrated that the desired challenge level of 100,000 to 400,000 spores (presented to an animal breathing at the rate of 4 L/min) was obtainable. It was also shown that cloud concentrations, as measured by 6 L/min all-glass impingers cutting off at 5 μ particle size, did not vary significantly over the length of the exposure section.

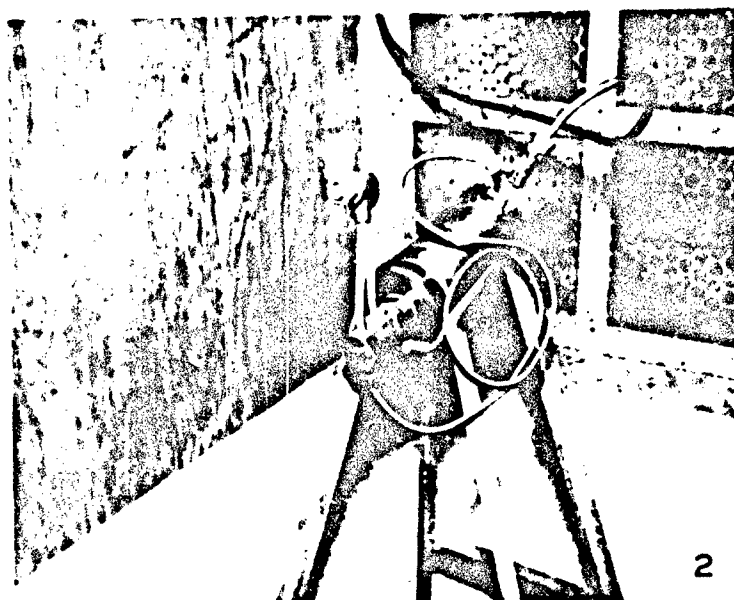


FIGURE 1. EXTERIOR VIEW OF WIND TUNNEL.
FIGURE 2. GENERATOR EMPLOYED.

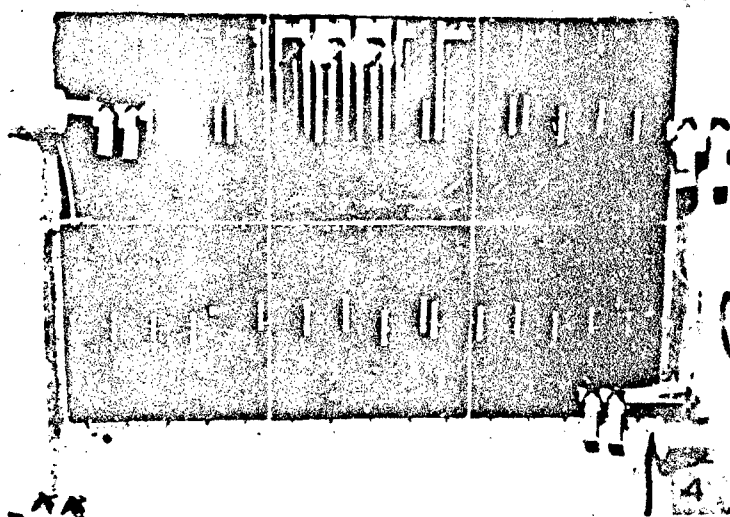
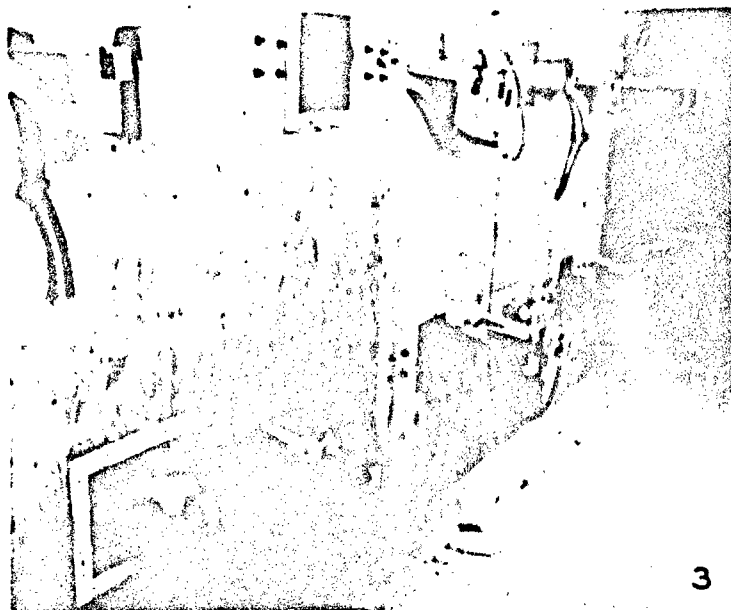


FIGURE 3. INTERIOR VIEW - ANIMALS & IMPINGERS IN POSITION.
FIGURE 4. DEEP-BED BACTERIAL FILTER ARRAY.

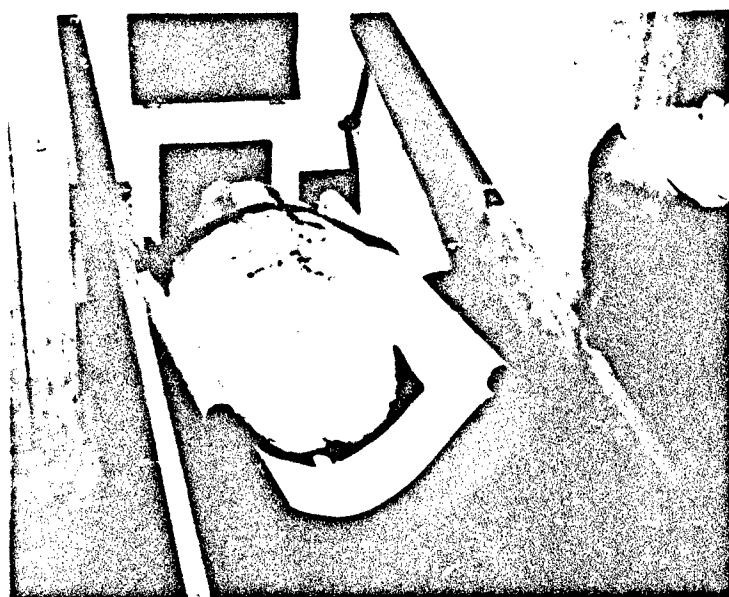
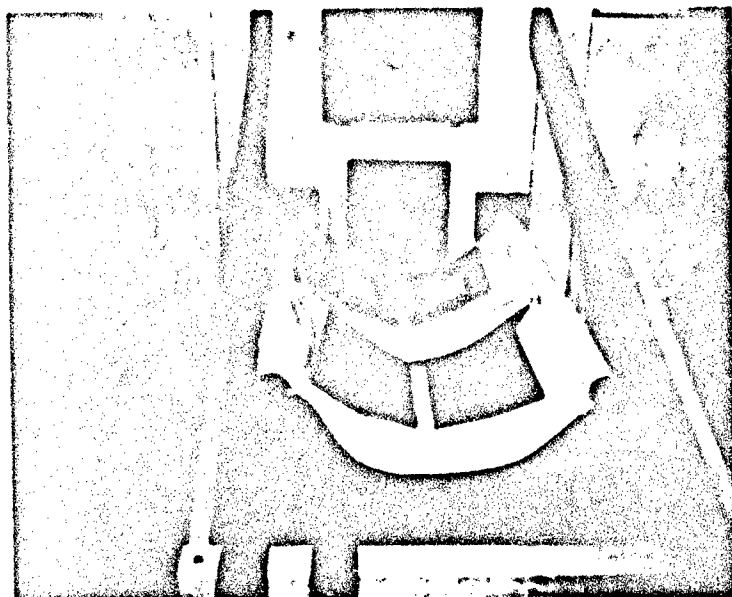


FIGURE 5. ANIMAL POSITIONING DEVICE.
FIGURE 6. SHEEP RESTRAINED IN POSITIONING DEVICE.

B. CHALLENGE STRAIN

The challenge strain of B. anthracis employed in these studies was a concentrated, phenolated spore suspension of Vollum-189 prepared July 5, 1957, by personnel of Medical Investigation Division, Fort Detrick. Four liters of suspension (in vaccine bottles packed in wet ice) were flown to DPG in September of that year. Spore counts for viability made at various times before and after shipment to DPG revealed no apparent reduction in viable cell count in one year's storage; the nominal count was 4×10^{10} spores/ml. Titrations in mice and guinea pigs showed no significant loss in virulence over the same period.

C. ANIMALS

Swine, monkeys, dogs, and sheep for the 1957 trials were procured by the Veterinary Division, Walter Reed Army Institute of Research (WRAIR) and air transported to DPG approximately three weeks prior to the September trial. Swine were of mixed breeds, weighing 73 to 95 pounds (avg. 84) in the first trial and 66 to 69 pounds (avg. 66.5) in the second. Swine had been immunized with live hog cholera vaccine (rabbit propagated) two weeks prior to shipment. Macaca mulatta monkeys weighed approximately 7 pounds. Dogs were mongrels, weighing 29 to 38 pounds (avg. 34); they had been immunized with chick embryo-propagated, live rabies vaccine and killed combined canine distemper-infectious hepatitis vaccine. Sheep were mixed breeds ranging in weight from 54 to 77 pounds (avg. 62); they had been immunized with a killed vaccine against Pasteurella multocida.

D. PATHOGENESIS STUDIES IN GUINEA PIGS (INTRACUTANEOUS CHALLENGE)

1. Trial I, August 19, 1957.

Each of 36 guinea pigs were inoculated intracutaneously in the right flank with 0.1 ml of a dilution of suspension containing approximately 256 spores. Three animals were sacrificed at each of the following hours post-challenge: 6, 18, 24, 30, 42, 48, 54, 66, 72, and 78. Sacrifice was by cardiac exsanguination of anesthetized animals. Five-tenths milliliter of blood was plated in two nutrient agar pour-plates the remainder being allowed to clot for future serological studies.

The right inguinal lymph node from two and approximately one-third of the spleen of three animals were emulsified in approximately 2.0 ml of nutrient broth in a tissue grinder. The node from the third animal and the remaining portions of spleens were preserved in formalin or quick frozen with carbon dioxide and alcohol for histologic examination. At autopsy other tissues including the site of inoculation, lungs, heart, mediastinum, liver, kidneys, and adrenal glands were fixed for further study.

Results of cultures of blood, spleen, and lymph node are shown in Table I.

Occasional organisms were recovered as early as six hours from a

lymph node near the site of inoculation. Recoveries were, in general, irregular until about 42 hours when at least one sample of each type yielded organisms. The pour-plates as used here were somewhat difficult to read; it was therefore planned to use blood agar streak-plates as well as pour-plates in Trial II.

TABLE I. ISOLATION OF B. ANTHRACIS FROM TISSUES OF INFECTED GUINEA PIGS AT VARIOUS TIME INTERVALS AFTER CHALLENGE

HOURS POST- INOCULATION	RECOVERY FROM SPECIMEN BY CULTURE		
	Heart Blood	Spleen	Right Inguinal Lymph node
6	- - ^{a/}	- - -	+ -
18	- - -	+ - -	- -
24	- - -	- - -	- -
30	- - -	+ - -	+ +
42	+ - -	+ - -	+ +
48	+ - -	+ + -	+ +
54	+ + +	+ + +	+ +
66	+ +	+ + +	+ +
72	+ + +	+ + -	+ +
78	+	+	+

(August 19, 1957)

a. Minus sign (-) indicates no isolation. Plus sign (+) indicates isolation of B. anthracis.

2. Trial II, September 9, 1958.

This trial was conducted not only to check the plating techniques but to attempt to detect circulating antigen in the blood. Twenty-one guinea pigs were inoculated as before. Sacrifices were made at 6, 12, 24, 30, and 36 hours, three animals each. One animal remaining alive after this time was sacrificed at 54 hours. Four plates were prepared as before and 0.5 ml was streaked on two blood agar plates. Two ml were placed in an equal volume of Alsever's solution^{2/} for preservation and study on circulating anthrax antigen.

B. anthracis was isolated from only one animal's blood during the first 36 hours. At 54 hours the surviving guinea pig had a positive blood culture. The two plating techniques gave identical results.

E. MISCELLANEOUS

Details of meteorological conditions, animal positioning and dose estimates are contained in DPG Technical Report, DPGR 232¹.

F. RESPIRATORY CHALLENGES

1. The first respiratory exposure of sheep, dogs, pigs and monkeys was conducted on September 24, 1957, using six of each. The second exposure of 15 sheep, 8 pigs, and 8 dogs was conducted on October 10, to extend the observations made on the first test group. Impinger estimates indicated that the same order of magnitude of dosage was obtained; results were therefore combined.

2. Sacrifice Method

All animals were sacrificed by exsanguination with a vacuum device under Nembutal anesthesia, to reduce the possibility of gross contamination of organs and the general environment by blood that might contain B. anthracis. At death the animals were immediately autopsied; specimens of viscera were aseptically selected and immediately forwarded to the bacteriology laboratory. Other specimens were collected and fixed in 10 per cent formalin for histologic examination. Selected specimens were also frozen in dry ice and alcohol for future studies.

3. Bacteriological Examinations

Blood specimens for culture were collected in vaccine bottles containing an equal volume of Alsever's solution. Two cultural methods were employed: (a) direct plating of 2 ml distributed equally among four bloodagar plates prepared from Difco blood agar base³ and containing 5 per cent defibrinated sheep blood and (b) the inoculation of 10 ml into a Castaneda-type bottle containing a diphasic tryptose medium^{3,4}. Plates and bottles were incubated at 37°C, 72 hours for plates and at least one week for bottles before discarding.

The following tissues and body fluids were collected aseptically at autopsy for bacteriologic examination:

Fluids: blood, bile, urine, and stomach and intestinal contents.

Swabs: bronchus, turbinate, and sinus.

Lymph nodes: tracheobronchial, hilar, mediastinal, submaxillary, cervical, and mesenteric.

Other tissues: lung, tonsil, meninges, liver, kidney, and spleen.

Samples of tissue provided for culture at autopsy were transferred from the sterile Petri dishes in which they were received to Tenbroek tissue grinders. Approximately 1.5 ml of broth was added and the tissue emulsified.

The contents of the grinders were removed with a syringe and divided between two blood or tryptose agar plates.

The inoculum was spread on all plates with conventional glass spreaders. The plates were then covered with sterile absorbent tops and allowed to dry in an upright position before inverting for incubation as described above.

4. Results.

a. Monkeys:

Morning temperatures were taken on 5 of 6 monkeys exposed. With the exception of a temperature of 104.0°F on the second-exposure day in one monkey all other temperatures were within normal limits (<103.0°F). The animals remained alert and active; their appetites remained good; they showed no prodromal signs of infection. All animals died suddenly with little or no warning of impending exitus. On day 3 two animals were dead in the morning and one in the afternoon; two were dead on the morning of day 4; and the remaining animal died on the fifth morning.

A limited autopsy performed on a day-3 monkey revealed subcutaneous and retroperitoneal hemorrhages and hemorrhagic mediastinitis. Most of the lymph nodes were hemorrhagic as were the tonsils. The spleen was not markedly enlarged. Blood films showed numerous anthrax bacilli.

b. Sheep:

Clinical observations: Respiratory anthrax in sheep was an acute febrile illness. Aside from fever, the animals that died appeared alert until a few hours before death. Their appetites remained good, rumination was uninterrupted, and the animals appeared neither lethargic nor hyperexcitable. The febrile pattern observed in this group of animals is shown in Figure 7. (For graphic reasons in this figure and other similar ones, temperatures between 104.2 and 105.0°F are shown as 105.0°F; 105.2 to 106.0°F as 106.0°F, and so on.) With one exception (No. 22), an animal found dead on the morning of day 2, all animals were febrile during the period of observation. There was usually a precipitous drop in body temperature shortly before death.

Bacteriological Examinations: It may be noted from Figure 7 that where demonstration of bacteremia was attempted, positivity was associated with the onset of fever. Two control animals in a limited therapy trial^{5/} were more exhaustively studied than those scheduled here for serial sacrifice; in one, No. 7, disease terminated in death at the end of the third day (Figure 8). The other animal, despite a positive blood culture late on day 2 and a protracted period of high fever, days 2 through 6, made an uneventful recovery with no specific or supportive therapy.

Table II shows the bacteriologic findings at time of sacrifice. Organisms were found in the lungs of all animals including the 15-day survivor. Generalized bacteremia was apparent at 28 hours post-exposure, but not at 15 days.

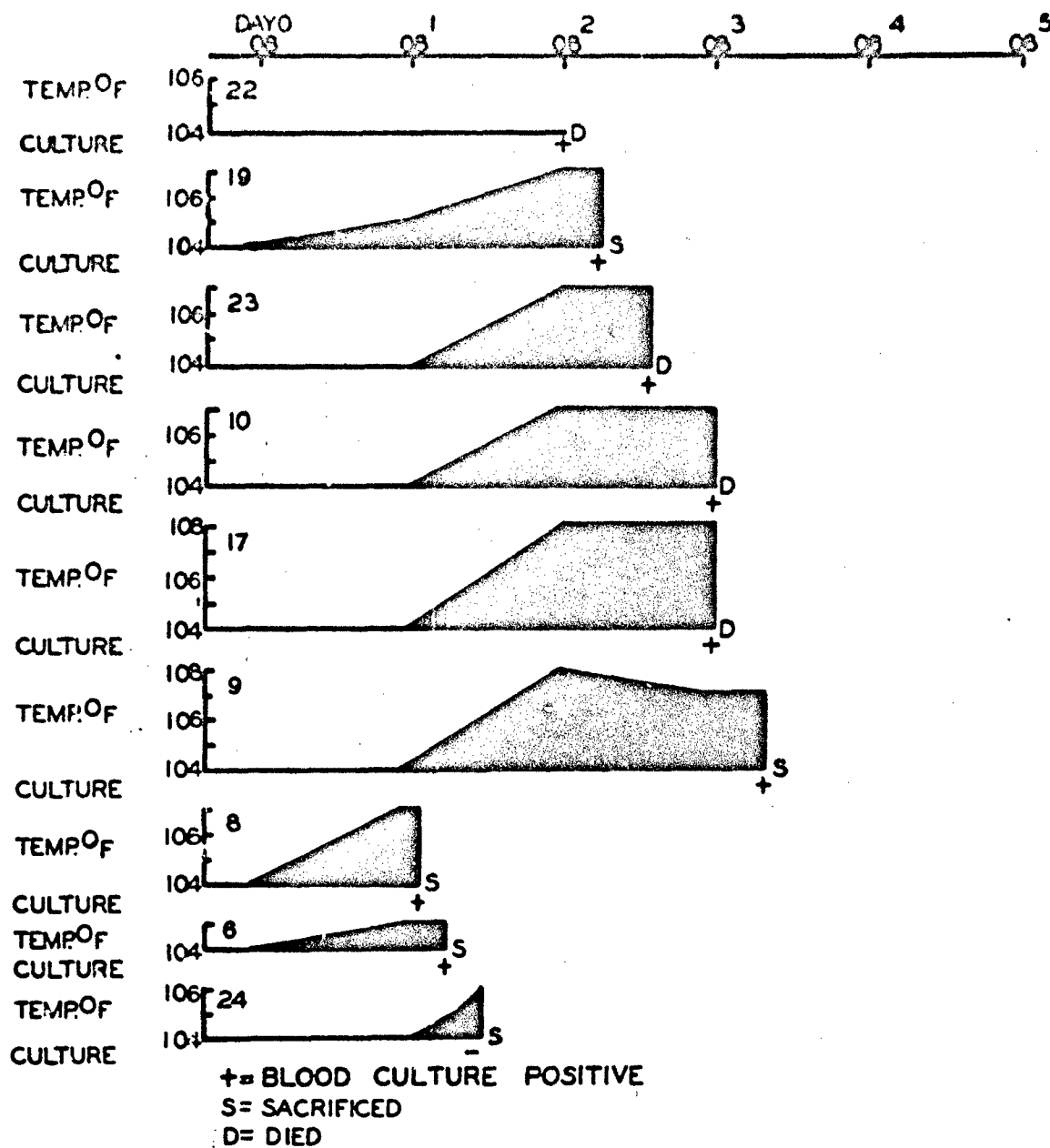


FIGURE 7. TEMPERATURE AND BLOOD CULTURE RESULTS IN SERIALY-SACRIFICED SHEEP. (SEPTEMBER 24, OCTOBER 10, 1957)

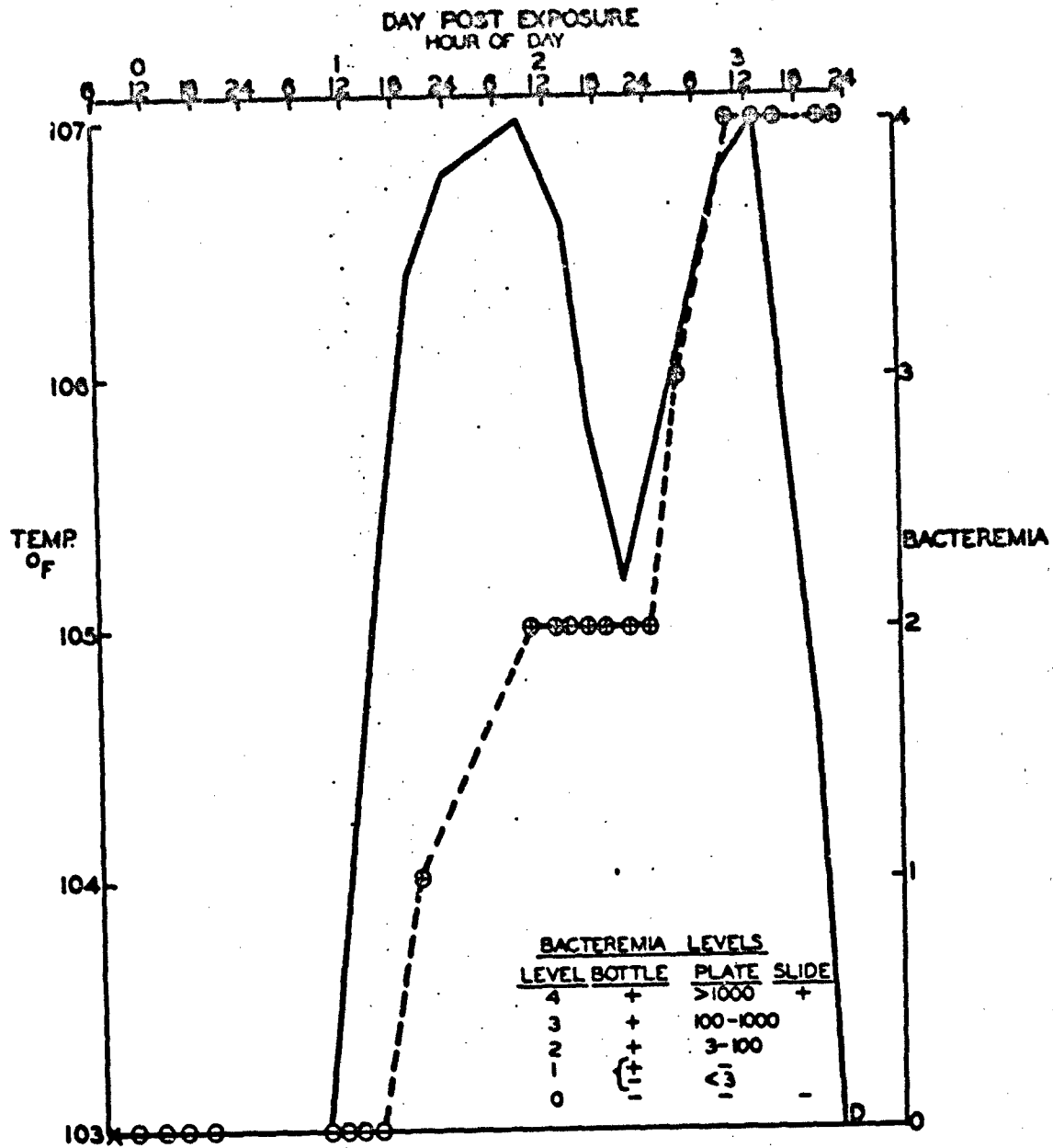


FIGURE 8. CORRELATION OF TEMPERATURE
AND BACTEREMIA (SHEEP 7)

TABLE II. DISTRIBUTION OF B. ANTHRACIS IN BLOOD AND TISSUES OF SHEEP AT TIME OF SACRIFICE^a/

CULTURE POSITIVITY FOR B. ANTHRACIS							
TIME OF SACRIFICE (hrs)	7	15	28	33	38	56	152 ^d
(SHEEP NO.)	(25)	(21)	(8)	(6)	(24)	(19)	(26)
LYMPH NODE							
Tracheal	-	-	+	+	+	ND ^c /	-
Hilar	-	-	+	+	+	ND	-
Mediastinal	-	-	+	+	+	ND	-
Cervical	-	+	-	+	-	+	-
Submaxillary	+	-	-	-	-	ND	-
TISSUE or FLUID							
Blood	-	-	+	+	Cd ^d /	+	-
Lung	+	+	+	+	+	ND	+
Tonsil	+	-	-	-	-	ND	-
Spleen	-	-	+	+	+	+	-
Liver	-	-	+	+	+	+	-
Kidney	-	-	+	+	-	+	-

a. 82-hour animal (No. 9) omitted as no bacteriological examination was done.

b. Day of sacrifice.

c. ND: not done.

d. C: contaminated.

Gross Pathology: The earliest time of sacrifice of an animal, not complicated by lesions of caseous lymphadenitis, was 28 hours; at this time mediastinal nodes were somewhat enlarged. At 33 hours these nodes were definitely prominent. This change was not present at 38 hours in an animal with caseous lymphadenitis. At 56 hours the mediastinal nodes were markedly enlarged and hemorrhagic but retained their identity; there were focal hemorrhages throughout the mediastinum and the neck strap muscles. At 82 hours all lesions were more advanced. In several animals the cervical nodes were prominent but the significance of this, if any, is not appreciated. In contrast

to the textbook descriptions of ovine anthrax the spleen was not enlarged nor were there diffuse hemorrhages.

Microscopic Pathology: Influx of macrophages into the sinuses of many of the lymph nodes was observed in the animals sacrificed early. At 56 hours and later there was virtual dissolution of the nodes with severe edema, some hemorrhage, disappearance of the lymphoid elements, and a moderately heavy infiltration of neutrophils and eosinophils. Clumps of fibrin were present and vegetative organisms were abundant throughout the section. (Figures 27 and 28) The mediastinal nodes at 56 and 82 hours were hemorrhagic.

At 56 hours there was a lesion in the lung involving the bronchioles and the adjacent pulmonary parenchyma which was similar to the late necrotizing lesion in the lymph nodes (Figures 29 and 30). There was marked dilation of the submucosa of the involved bronchioles; the epithelium of the bronchus was intact. However, the submucosa was swollen and filled with neutrophils, clumps of fibrin, eosinophils, and numerous vegetative organisms. This process extended into the adjacent pulmonary parenchyma. Masses of fibrin were seen in adjacent blood vessels. The impression was that the lymphatics in the walls of the bronchi were the focal point of this lesion, leaving the bronchial epithelium and muscle layers intact. The normal architecture of the adjacent pulmonary parenchyma was completely destroyed. Hemorrhagic enteritis as seen in the intestines of sheep No. 19 was probably a terminal phenomenon, as was also the myocardial degeneration.

There was a marked influx of neutrophils into the spleen of sheep No. 24, sacrificed at 38 hours. Acute lysis was noted in the spleen of animal No. 9 sacrificed at 82 hours.

Using the Brown and Brenn (B&B) stain, masses of bacilli were demonstrated in the hilar lymph nodes of the 28- (Figures 31 and 32) and 38-hour-sacrifice animals. No bacilli could be found in the lung sections of these animals.

Incidental lesions of caseous lymphadenitis and lungworm infection were present in the lungs and lymph nodes of some of these sheep. There was also acute, patchy, peribronchiolar pneumonia, considered to be nonspecific.

Changes in the renal epithelium were observed in four animals, the 28-, 33-, 56-, and 82-hour sacrifices. In the convoluted tubules there was modest degeneration of the epithelium which was swollen and vacuolated; cell outlines were indistinct; in some cases the cells were completely detached from their basement membranes and necrotic. Amorphous, eosinophilic material filled the lumina of many of these tubules. These changes might represent moderate post mortem degeneration; however, all these animals were autopsied immediately after they were sacrificed. These findings were considered non-specific degenerative changes. "Lower nephron nephrosis" was not recognized.

Gross and Microscopic Pathology of a Sheep Sacrificed Day 15:
Animal No. 26 survived an acute febrile illness with an associated bacteremia on day 2. At autopsy the posterior mediastinal and one of the cervical lymph

nodes were enlarged. There were numerous reddish-purple patches of consolidation approximately 4 cm in diameter immediately beneath the visceral pleura. These, on cross-section, appeared to be firm and calcified. No other significant lesions were noted grossly. Lesions of caseous lymphadenitis were seen microscopically in the lungs and hilar and mediastinal lymph nodes. In one of these latter lesions, Gram-positive bacilli were demonstrated by B&B staining, although none were recovered from either the mediastinal or hilar lymph nodes on culture. Acute, patchy, peribronchiolar pneumonia was observed in several of the sections; B&B stains failed to reveal the presence of any organisms within these lesions. This lung lesion was considered incidental and not directly related to the infection this animal had survived.

Table III summarizes significant findings:

c. Swine:

Clinical Observations: - A total of 14 swine were challenged. Eight animals showed fever of varying intensity during the period of observation (Figure 9); there were no other clinical signs of illness. Appetite and attitude remained normal in the febrile periods.

Bacteriological Examinations: - Isolations from lungs were made consistently through day 10 with one exception, a 6-day sacrifice. Other cultures were negative with the exception of an isolation from a tracheobronchial lymph node on day 6.

Gross Pathology: - Significant gross lesions were primarily found in the lungs and pulmonary lymph nodes. There was marked enlargement of hilar and tracheobronchial nodes in one animal sacrificed on day 6 (Figure 10); no discrete lesions were involved; cut surfaces presented a uniform homogeneous appearance. Discrete, hemorrhagic, pulmonary lesions were observed in two animals, the 8- (Figure 11) and 10-day sacrifices; in one, hemorrhage was distinctly peribronchial; in the other, the lesions were round, circumscribed, solitary nodules which were not encapsulated. The cut surface was dark and firm; the lesions were located in the pulmonary parenchyma. Other lesions were present in the lungs but were common in hogs under normal conditions. Hemorrhagic mediastinitis observed in three animals was due to trauma associated with bleedings from the anterior vena cava. Roundworms (Ascarids) were present in the intestinal tract of many of the swine.

Microscopic Pathology: - Significant lesions were essentially confined to lungs and lymph nodes. Intense hemorrhagic, fibrinous pneumonia was observed in three animals, 6-, 8-, and 10-day sacrifices (Figures 15, 16, 17, 19, 20, 24, and 25). Two such lesions (in 6- and 8-day sacrificed animals) were immediately adjacent to bronchi. They were discrete with definite boundaries, but non-encapsulated. The core of hemorrhage was surrounded by masses of fibrin, which occluded alveoli, bronchioles, and vessels, and an intense cellular infiltrate. The hemorrhagic lesion was suggestive of an infarct although not in the true pathological sense of being caused by an embolus. Associated with the pneumonia were markedly dilated lymphatics in the septa of the lungs. In these lymphatics there were masses of fibrin and

TABLE III. PATHOLOGY AND BACTERIOLOGY OF SEXUALLY-SACRIFICED SHEEP

SHEEP (ACC.) MONS. SEX NO.	Pathology	LUNGS		BACTERIOLOGY		LYMPHATIC SYSTEM		OTHER	
		Microscopic	Cult.	Micro.	Mode	Pathology	Microscopic	Cult.	Pathologic Diagnosis
1 (127)	WNL	Caseous lymphadenitis	+	ND	Mediastinal	WNL	Influx of macro- phages	-	Tonsill: normal
2					Cervical Submaxillary	Enlarged (?)	-	+	
10 (128)	Consolidation, patchy edema	Pneumonia, patchy, bron- chiolar	+	ND	Mediastinal	WNL	Scarring, influx of macrophages	-	Tonsill: enlarged
2		Caseous lymphadenitis			Cervical	Enlarged, focal hemor- rhage	Same	+	
28 (129)	Edema	Pneumonia, patchy, bronchiolar	+	-	Mediastinal Tracheobr. Hilar	Enlarged (?)	Old scarring edema Same	+	Tonsill: enlarged
2					Cervical	Enlarged	Hyperplasia, influx of macrophages	+	Nephroses (?)E/ Bacteremia Infarct, liver
33 (130)	WNL	-	+	ND	Mediastinal Tracheobr. Hilar	Enlarged, congested	Congestion, erythro- phagocytosis	+	Bacteremia Nephroses (?)
2					WNL	WNL	-	+	
28 (131)	"BB"-size nodules	Caseous lymphadenitis	+	-	Mediastinal Tracheobr. Hilar	WNL	Caseous lymphadenitis Same	+	Bacteremia Nephroses, focal, hepatic
2					WNL	WNL	-	+	Myocarditis, focal Splenitis, acute
36 (136)	WNL	Bronchitis, acute, necrotizing, focal	ND	+	Mediastinal Cervical	Enlarged, hemorrhagic	Lymphadenitis, acute necrotizing, lysing	ND	Mediastinitis, hemorrhagic, acute Enteritis, hemor- rhagic (?)
1						Enlarged	Same	ND	Nephroses, subepi- cardial Nephroses (?)
8 (137)	Consolidation, patchy	Pneumonia, acute, patchy	ND	+	Mediastinal Cervical	Enlarged, hemorrhagic	Lymphadenitis, acute necrotizing, lysing	ND	Myocardial degeneration Mediastinitis, hemorrhagic, severe
1						Same	Same	ND	Bacteremia, terminal Nephroses (?)
11th Day (137)	Consolidation, focal, patchy, multiple	Pneumonia, acute, patchy Caseous lymphadenitis	+	-	Mediastinal Hilar	Enlarged WNL	Caseous lymphadenitis Same, scarring	-	Hemorrhages, peri- toneal, mesenteric Splenitis, lysing

a. WNL. No visible lesions.
 b. Lesions of caseous lymphadenitis in the lungs are found in peribronchial lymphocytic aggregates.
 c. ND. Not done.
 d. Microscopic examination positive for bacilli.
 e. Nephroses is used here merely to indicate degenerative changes of the tubular epithelium, which are not considered specific and are not "lower nephron
 nephroses".
 f. C. Contaminated.

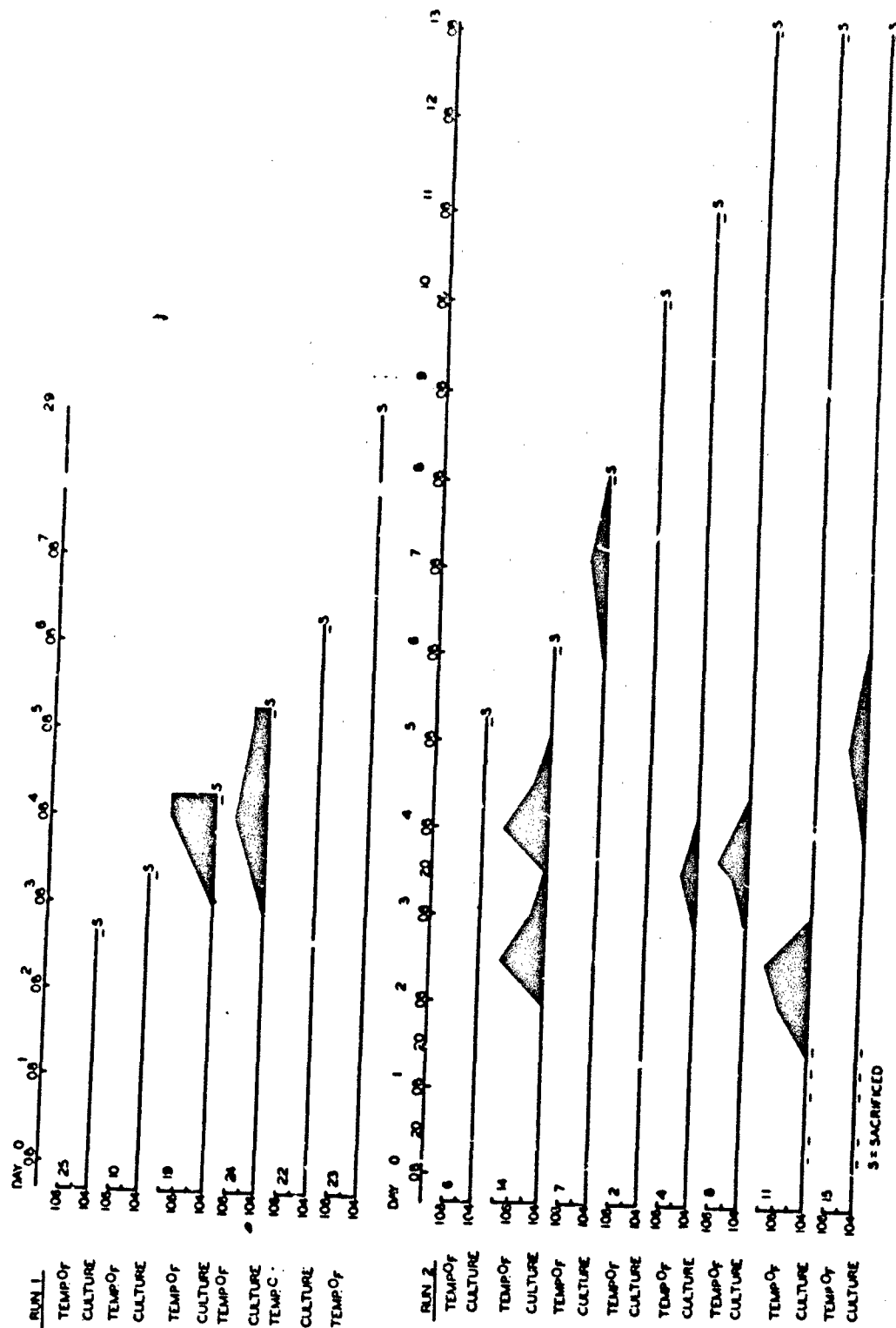


FIGURE 9. TEMPERATURES AND BLOOD CULTURE RESULTS IN SERIALY-SACRIFICED SWINE. (SEPTEMBER 24, OCTOBER 10, 1957)



FIGURE 10. 8 DAY PIG NO. 7 (ACC 136). SECTION OF LUNG SHOWING HEMORRHAGIC LESION.
FIGURE 11. 8 DAY PIG NO. 7 (ACC 136). ENLARGED HILAR & TRACHEOBRONCHIAL NODES.

bacilli, morphologically compatible with B. anthracis. The cellular response consisted of large macrophages filled with cellular debris, lymphocytes, neutrophils, and some eosinophils. Occasional focal collections of neutrophils were seen in some of the alveoli. The pleura was markedly thickened and contained a heavy cellular infiltrate and fibrin. Thrombosed vessels were seen in the areas of hemorrhage. Bronchioles were completely filled with fibrin masses and cellular debris. Other lesions, primarily bronchopneumonic, were seen in these animals but were different in nature and were considered as incidental, resulting from a non-specific virus pneumonia and the migration of lung worm larvae.

The intense "hemorrhage infarct" type of lesion and its surrounding acute fibrinous pneumonia were considered to be significant lung lesions resulting from the respiratory exposure to B. anthracis.

Reactive hyperplasia of the lymphoid elements was seen in lymph nodes. These changes were essentially those of enlargement of the germinal centers with an increase in the numbers of mitotic figures. There was depletion of the mature lymphocyte population of the follicles and an increase in large, immature blast forms (Figures 18 and 26); edema was present. Necrosis was prominent in the pulmonary nodes of those three animals with hemorrhagic and fibrinous pneumonia (Figure 21). The same reactive changes described here were also seen in the lymphatic elements of the spleen.

Scarring of the lymph nodes was observed, evidenced by varying degrees of proliferation of young fibroblasts, along with a light to moderate infiltration of eosinophils. These lesions were attributed to the migrating lung worm larvae which travel through the lymphatic system to their final site in the bronchi. Table IV summarizes significant findings.

d. Dogs:

Clinical Observations: - A total of 14 dogs were exposed. Three of these had significantly elevated temperatures at varying times during the period of observation; four had a single minimally elevated temperature. No other signs of illness were manifested in them or in the animals that remained afebrile. Temperature responses are shown in Figure 12.

Bacteriological Examinations: - Isolations were frequently made from the lungs and in many instances from pulmonary lymph nodes.

Gross Pathology: - Significant changes were found primarily in the lungs and hilar and tracheobronchial lymph nodes. Beginning with the sixth day of sacrifice enlarged pulmonary lymph nodes were observed (Figure 13). Discrete, unencapsulated, dark, firm pulmonary lesions were observed in three animals, those sacrificed at 8, 10, and 11 days. The lesion in the 8-day sacrifice dog was large and immediately adjacent to a bronchus (Figure 14). Large tonsils were observed in several dogs; however, this finding is one frequently observed in a "normal" canine population at autopsy. Pin-head size, subcapsular, discrete, white spots were seen in the kidneys. Intestinal parasites were present in most dogs.

TABLE IV. PATHOLOGY AND BACTERIOLOGY OF SERIALY-SACRIFICED SWINE

Pig (ACC.)	Days Run	LUNGS		LYMPH NODES		OTHER	
		Pathology	Bacteriology	Pathology	Pathologic	Diagnosis	Cult.
		Gross	Micro.	Gross	Microscopic		
24	25	Pneumonia, focal	+	Hilar	Hemorrhage		
(117)	1	Atelectasis	+	Gastric	Same		NO
		Lung worms		Hepatic	Same		NO
34	10	Consolidation, Emphysema	+	Tracheobr.			
(119)	1	Atelectasis		Mediastinal	Hemorrhage		-5/
4-1/3	19			Hilar	Hemorrhage		-5/
(122)	1			Tracheobr.			
		Lung worms		Mediastinal	Hemorrhage		
		Pneumonia, focal	+	Hilar	Same		
				Hepatic	Same		
5	25		+	Tracheobr.	Same		
(124)	1			Mediastinal	Hemorrhage		
		Lung worms		Hilar	Enlarged 1x		
		Pneumonia, severe, parasitic	+	Tracheobr.	Same		
5 1/2	6	Edema, mild	+	Mediastinal	Enlarged (1)		
(133)	2			Hilar	Same		
		Lung worms			Diffuse		
		Pneumonia, parasitic	+		scarring		
		Giant cells			eosinophilia		
6	22	Emphysema	+	Mediastinal	Hemorrhage		
(126)		Atelectasis		Hilar	Same		
				Tracheobr.	Same		
6	14	Atelectasis, Infarct, discrete, hemorrhage	-	Mediastinal	Enlarged 2x		
(134)	2	Pneumonia, acute, Lung worms, fibrous (pneumonia)		Hilar	Same		
				Tracheobr.	Same		
8	7	Discrete nodules, dark centers	+	Cervical	Hemorrhage		
(136)	2			Mediastinal	Enlarged 3x		
				Tracheobr.	Same		
				Hilar	Enlarged 2x		
10	2	Discrete nodules, dark, multiple	+	Mediastinal	Enlarged 3x		
(140)	2			Hilar	Same		
				Tracheobr.	Same		
11	4	Consolidation, patchy	-	Mediastinal	Enlarged 2x		
(142)	2			Hilar	Same		
				Tracheobr.	Same		
13	8	Emphysema	-	Mediastinal	Enlarged 2x		
(143)		Atelectasis		Hilar	Same		
				Tracheobr.	Same		
20	23	Consolidation, patchy	-	Mediastinal	Enlarged 2x		
(144)	1			Hilar	Same		
				Tracheobr.	Same		

a. MD: Not done.
b. MVL: No visible lesions.
c. Microscopic examination negative for bacilli.
d. Hemorrhage in the mediastinum in the pig is attributed to bleedings from the anterior vena cava.
e. Diffuse scarring of the lymph nodes is attributed to migration of lung worm larvae through the lymphatic system.

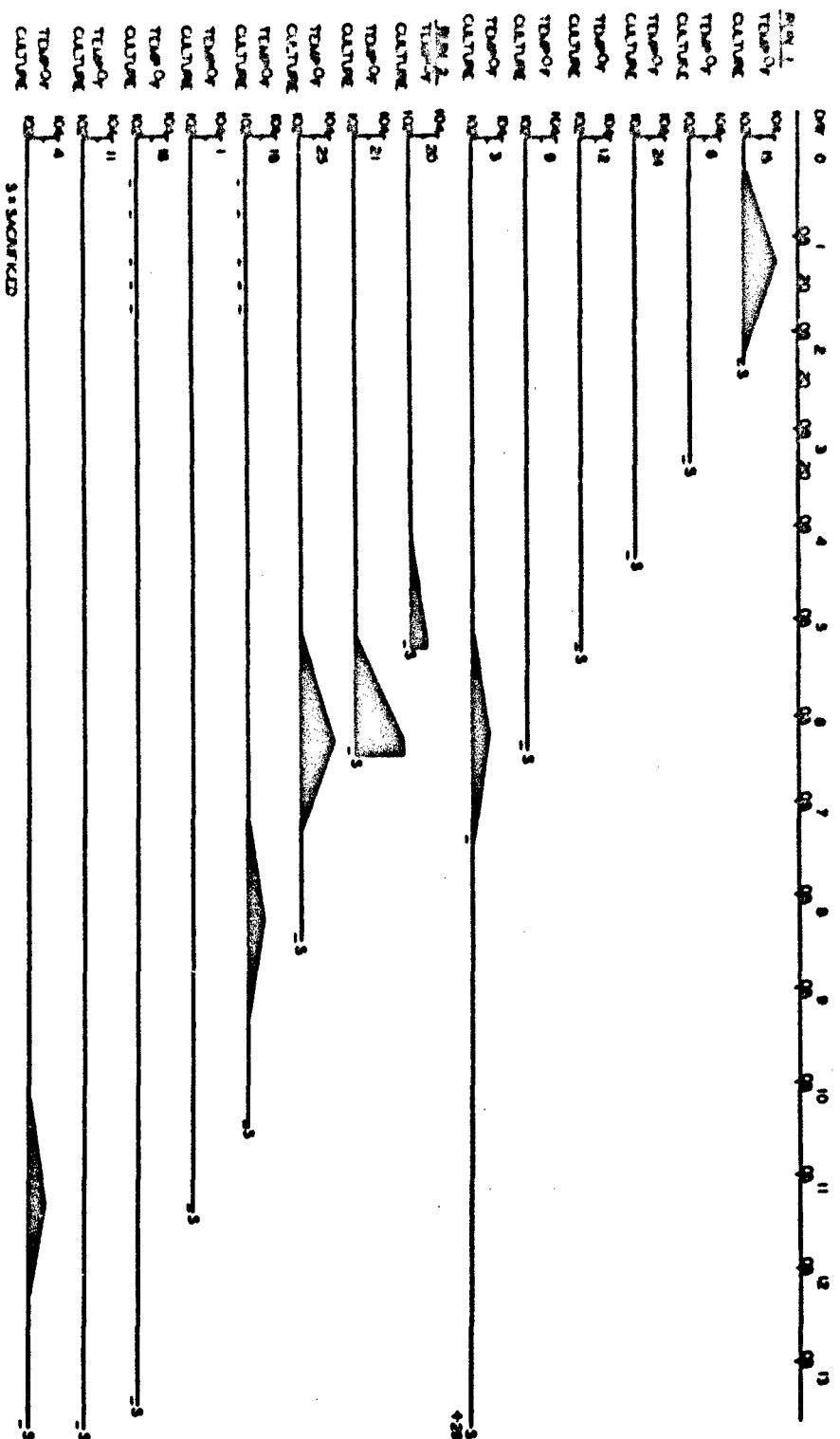


FIGURE 12. TEMPERATURES AND BLOOD CULTURE RESULTS IN SERIALY-SACRIFICED DOGS.
(CONTINUED 24, OCTOBER 19, 1957)

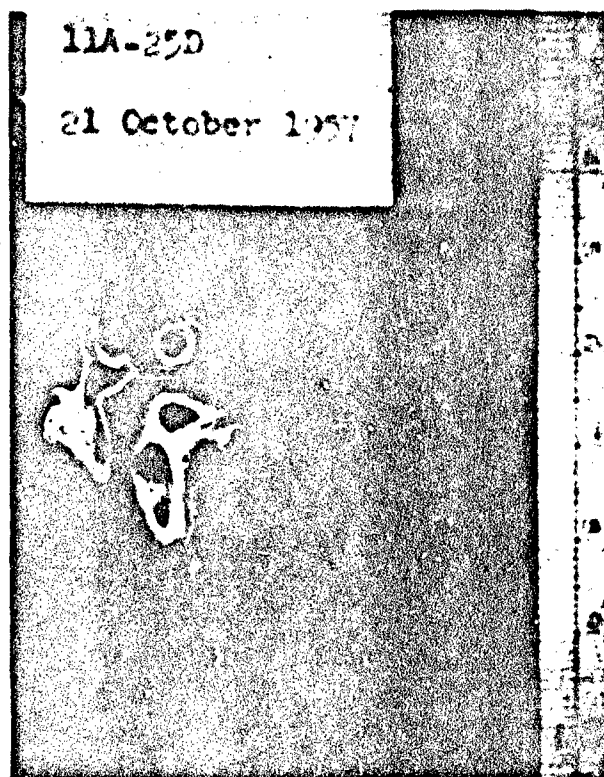
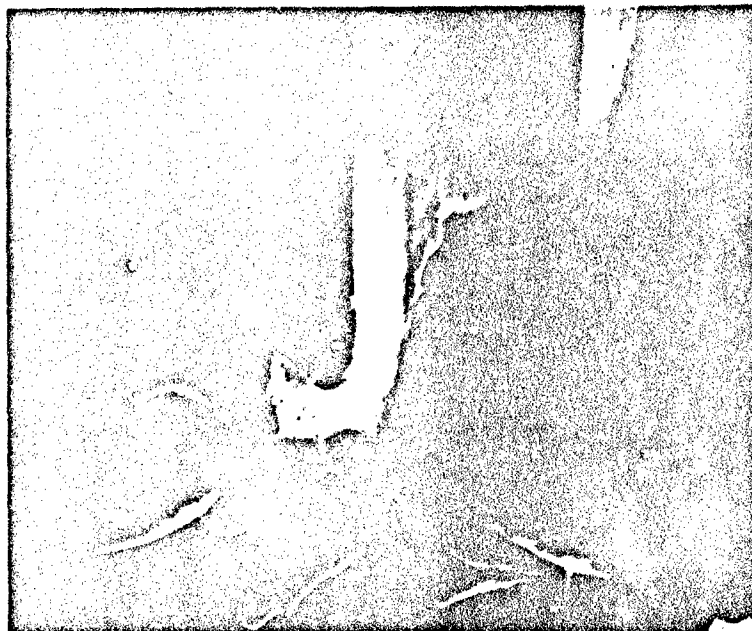


FIGURE 13. 8 DAY DOG NO. 21 (ACC 135). ENLARGED HILAR & TRACHEOBRONCHIAL LYMPH NODES.

FIGURE 14. 8 DAY DOG NO. 25 (ACC 137). SECTION OF LUNG SHOWING LARGE HEMORRHAGIC LESION.

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Microscopic Pathology: - Significant lesions were confined essentially to lungs and lymph nodes. Lung lesions in two animals, the 8- (Figure 22) and 11-day sacrifices, were similar to those described in the pig lung. The massive hemorrhages were suggestive of hemorrhagic infarct. Masses of fibrin and a heterogeneous cell population consisting of lymphocytes, neutrophils, plasma cells, and large macrophages surrounded the zones of hemorrhage. Bronchioles and vessels in the involved area were filled with masses of fibrin.

Granulomas in the lungs of several of the animals were attributed to parasites. Patchy pneumonia with heterogeneous cell populations, including large macrophages and multinucleated giant cells, was observed in two animals, 10- and 13-day sacrifices. The true significance of these pneumonic lesions is not known since they might represent later stages of the hemorrhagic lesions previously described which were undergoing organization, or they might be incidental.

The changes in pulmonary lymph nodes, beginning with the fifth day were essentially those of reactive hyperplasia. Enlarged germinal centers were prominent; they possessed increased numbers of mitotic figures, depletion of mature lymphocytes in the follicles, and increased numbers of large immature blast forms (Figure 23). In one dog (a 6-day sacrifice) frank necrosis was observed in the pulmonary lymph nodes with marked neutrophilic infiltration. There were no significant pulmonary lesions in this animal; organisms were demonstrated by culture but not histologically in the lymph nodes.

Lymphatic elements of the spleen showed the same changes as lymph nodes, although there was no necrosis.

Bacteriological and pathological findings in these dogs are shown in Table V.

III. DISCUSSION

A. MONKEYS

No generalizations are warranted from the limited observations. The phenomenon of sudden death in the absence of prodromata merits critical investigation. The apparent absence of fever sets monkey respiratory anthrax apart from the infection as seen in other animals. It appears that monkeys ignore the infection until overwhelmed by it.

B. SHEEP

One of the principal problems in the study of respiratory anthrax in sheep lies in the variation in responses between animals to a given challenge. Because of this, serial sacrifices must be interpreted with caution, as the probable death or survival of these animals cannot be predicted. From the standpoint of illness and recognition of disease, it appears that fever is the only reliable criterion of infection. The bacteriologic techniques employed seldom permit the demonstration of bacteremia before an animal dies, or recovers.

TABLE V. PATHOLOGY AND BACTERIOLOGY OF SERIALY-SACRIFICED DOGS WITH RESPECT TO LUNGS

DOGS			LUNGS		LYMPH NODES			OTHER	
DAYS	RUN NO.'s	Pathology	Gross	Microscopic	Bacteriology	Name	Pathology		Pathologic
							Gross	Microscopic	
3	6	NVL	-	-	+	Mediastinal	Hemorrhage	Cult.	Diagnosis
	(118)					Tracheobr.		-	Tonsillitis; hemorrhage, petechiae
	1					Hilar		+	
+	26	NVL	Pneumonia, focal		-	Hilar			Liver; Macrosis, focal
	(121)		Hemorrhages, interstitial, focal		MD	Tracheobr.		+	
	1					Cervical	Enlarged 1+	-	Lymphoid hyperplasia
	1					Mesenteric	Same, coagulated	-	Same, erythrophagocytosis
5	12	NVL	Hemorrhage, focal		+	Mediastinal		+	Hepatitis, chronic
	(123)		Granulomas, parasitic		-	Hilar		-	Tonsillitis, petechial
	1					Tracheobr.		+	
	2					Gastric	Congestion 1+	-	
5	20	NVL	Granulomas, parasitic		+	Mediastinal	Same	MD	
	(132)				-	Hilar	Reactive, loss of mature cells	MD	Tonsil: hypertrophy, hyperplasia
	2					Tracheobr.	many blast cells	MD	
6	9	NVL	Pneumonia, focal		+	Hilar	Enlarged 1+	+	Tonsillitis; enlarged;
	(125)		Granulomas, parasitic (nematodes)		-	Tracheobr.	Lymphoid hyperplasia	+	petechiated
	1						Same	+	
6	21	NVL	-		+	Mediastinal	Edema, hyperplasia	+	Tonsil: hypertrophy, hyperplasia
	(135)				-	Hilar	Reactive hyperplasia	-	Kidney: Acute glomerulonephritis
	2					Tracheobr.	Acute lymphadenitis	-	Tonsil: petechial hemorrhage
8	25	Discrete, hemorrhagic	Infarct, hemorrhagic		+	Mediastinal	Enlarged 2+	-	
	(137)		Pneumonia, acute fibrinous		-	Hilar	Enlarged 3+	-	
	2					Tracheobr.	Enlarged 3+	-	
10	18	Discrete, dark nodular lesion	Pneumonia, acute, influx of macrophages		+	Hilar	Enlarged 2+	-	
	(141)				-	Tracheobr.	Reactive hyperplasia	-	
	2						edema, fibrin	-	
11	1	Discrete, dark nodular lesions	Pneumonia, hemorrhagic fibrinous, acute		+	Mediastinal		+	
	(143)				-	Hilar		-	
	2					Tracheobr.	Enlarged 2+	-	
13	16	NVL	Pneumonia, focal parasitic (?)		+	Hilar	Enlarged 1+	+	Tonsil: petechial hemorrhage
	(147)				-	Tracheobr.	Same	+	
	2						Lymphoid hyperplasia, mild	-	
29	3	NVL	-		-	Hilar		-	Tonsil: petechial hemorrhage
	(149)				MD	Tracheobr.	Enlarged	-	
	2						Lymphoid hyperplasia, mild	-	

a. NVL No visible lesions.

b. Microscopic examination for bacteria negative

c. MD Not done

The presence of caseous lymphadenitis lesions also makes interpretation of serial-sacrifice data difficult, as there is a strong suggestion that they markedly modify the pathogenesis of this disease. Lymph node alterations were noted in animals sacrificed at 28- and 33-hours, both being free of caseous lymphadenitis. The histologic demonstration of masses of bacilli in the node at 28 hours, unassociated with marked histologic change, pointed to the role of the nodes as possible "breeding grounds" for the organisms. The 36-hour-sacrifice sheep had extensive caseous lymphadenitis. Animals sacrificed at 56 and 82 hours had florid, mediastinal lesions of anthrax with no caseous lymphadenitis. The survival of sheep No. 26 to day 15 after a period of fever and bacteremia lends support to the interference hypothesis. Extensive caseous lymphadenitis was observed at autopsy in this animal.

No definite statement can be made concerning the time of appearance of overt mediastinal lymphadenopathy; based on two animals, the 28- and 33-hour sacrifices fever and bacteremia precede adenopathy. Reasoning from the findings in a fatal case (No. 7)^{5/}, bacteremia may remain at a modest level for some 24 hours before becoming massive. Delineation of the interrelationship between time of mediastinal lymph node involvement and onset of fever with or without bacteremia will require additional studies employing appropriate x-ray techniques together with bacteriological and pathological examinations.

The temporal relationship of the necrotizing lesion of the wall of the bronchus to the disease process in Sheep No. 19 is not known. It is not considered to be a primary lesion.

The mechanism of death from anthrax infection in sheep remains unanswered. The possible interference between pre-existing caseous lymphadenitis and the respiratory challenge with B. anthracis requires further investigation.

C. SWINE AND DOGS

Swine and dogs handled anthrax infection well; the infection was sub-clinical, or extremely mild. No bacteremia was detected. However, the enlargement of the pulmonary lymph nodes in some of these animals was such that x-ray confirmation of infection should have been obtainable.

The hemorrhagic and fibrinous pulmonary lesions may represent an attempt on the part of the host to "wall off" the intruder. The exact pathogenesis of this lesion is not understood, nor is it known what its temporal relationship is to the lymph node changes. Which came first remains an unanswered question; they probably began about the same time, but proof is lacking. It is believed that the masses of fibrin seen in these lung lesions represent the host's response to the challenge. The reactive hyperplasia of the lymph nodes is further evidence of the response of the host and his ability to handle the infection.

Lung worm infestation of swine further confuses the picture, particularly since the changes produced by these parasites and their migrations affect the respiratory and lymphatic systems. All pneumonic lesions which could have been associated with migrating larvae were considered incidental; diffuse

scarring of the lymph nodes was discounted for the same reason. In view of this, one has to question the validity of the gross findings in the enlarged hilar and tracheobronchial nodes.

The pulmonary and lymphatic lesions in dogs are considered significant.

Failure to isolate B. anthracis in all but one instance from hilar and tracheobronchial lymph nodes of swine, was in marked contrast with numerous isolations made from these nodes in dogs. This is difficult to explain since the natural disease in swine is frequently confined to the tonsils and pharyngeal lymph nodes.

D. GENERAL

Spores were not identified histologically in any lung sections, but exhaustive searches were not made. Bodies resembling spores were seen in sections of lymph nodes of sheep but proof is lacking.

The "resistant" dog and pig limit the process to the mediastinal area and maximum intensity of the pathologic lesions is attained after a much longer interval than in the "susceptible" sheep (or guinea pig). In this connection, studies on the anatomical distribution of lymphoid tissue in these animals may be of importance; grossly, dogs and pigs seem to have more lymphoid tissue distributed throughout lung parenchyma than sheep.

These introductory examinations of animal anthrax have been almost purely taxonomic; this approach obviously requires more exploitation. The role of phagocytosis has not even been cursorily explored. The pathogenesis in "immunized susceptibles" is unknown, as are the alterations introduced by chemotherapy in the fully susceptible species. With the extension of these studies biochemical and metabolic examinations should be included. Once some insight is gained into the basic host-parasite relationship its further manipulation by coincident or preceding virus infections is obviously warranted. The study of swine influenza and anthrax appear to merit consideration. Efforts to suppress "natural resistance," as well as artificial immunity in the "susceptible" animal, by pre-occupation of the immune mechanism by systemic viral infections should also provide an interesting approach.

SUMMARY

Investigations were conducted to explore the pathogenesis of respiratory anthrax in sheep, dogs, and swine. It was shown that dogs and swine, so-called "resistant" species, could be infected by the respiratory route and that the disease was comparable to that in sheep, differing only in severity. The principle areas involved were lymph nodes draining the lungs and their parenchyma. There was a strong suggestion that the lung lesions began in the lymphatics along the bronchi and major vessels although the temporal relationship is not clear. It was further suggested that intercurrent infections involving the systems of apparent primary concern, i. e. caseous lymphadenitis in sheep, lungworm infestation in swine, and parasitic granulomata in dogs,

might materially modify the spread of B. anthracis in favor of the host. The suggestion of interference is in line with somewhat comparable studies in guinea pig anthrax.

ACKNOWLEDGMENT

Contributions to this study Part 1 and Part 2 are greatly appreciated:

Dr. Byrd Olsen, for supervision of operations at DPG.

Dr. George C. Wright, Medical Investigation Division, Fort Detrick for B. anthracis slurry and protective antigen.

6570th Test Group (CalC & Ord) for air shipments.



FIGURE 15. 6 DAY PIG NO. 14 (ACC. 134). LUNG - MEMORRHAGIC "INFARCT" IMMEDIATELY ADJACENT TO A BRONCHUS CONFINED BY SEPTA. H & E 12X.

FIGURE 16. 6 DAY PIG NO. 14 (ACC. 134). LUNG - AREAS OF MEMORRHAGE & ACUTE FIBRINOUS PNEUMONIA. H & E 75X.

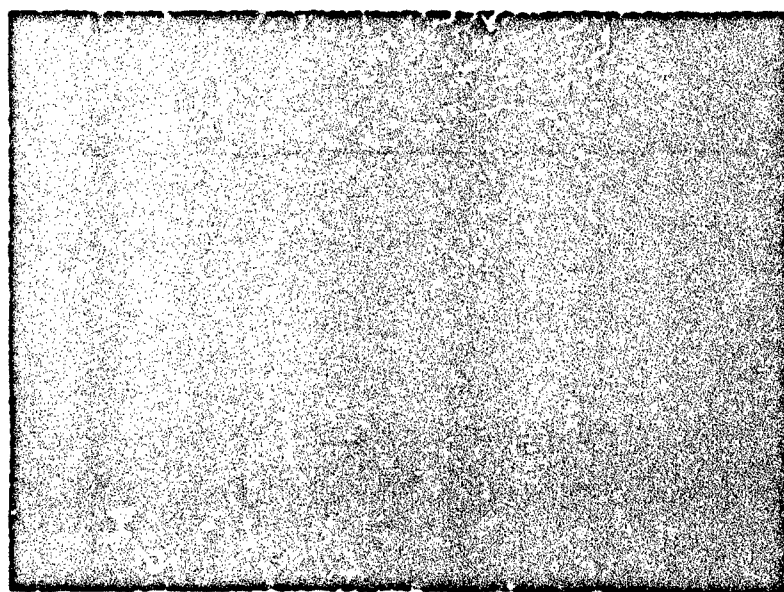
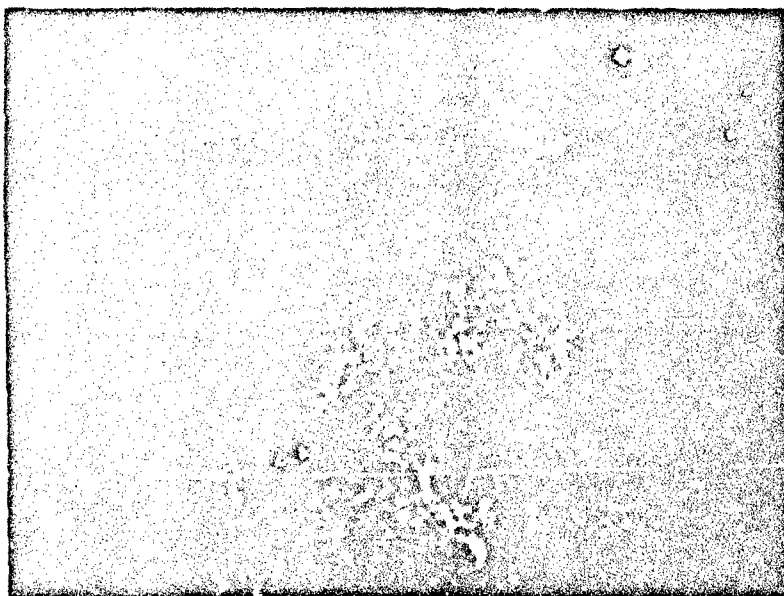


FIGURE 17 8 DAY PIG NO.14 (ACC 134). LUNG - MASSES OF FIBRIN & CELLS.
H & E 210X.

FIGURE 18. 8 DAY PIG NO.14 (ACC 134). HILAR LYMPH NODE - DEPLETION OF
MATURE LYMPHOCYTES. H & E 200X.



FIGURE 19. 8 DAY PIG NO. 7 (ACC 136). LUNG - HEMORRHAGIC PNEUMONIA & DILATED LYMPHATIC FILLED WITH MASSES OF FIBRIN & CELLS. H & E 86X.

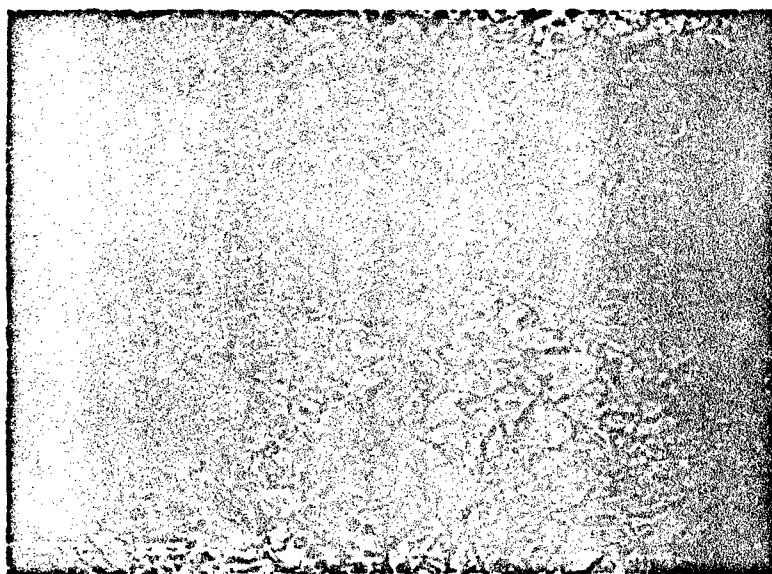


FIGURE 20. 8 DAY PIG NO. 7 (ACC 136). LUNG - MASSES OF BACILLI IN DILATED LYMPHATIC: SHOWN ABOVE. B & B 400X.

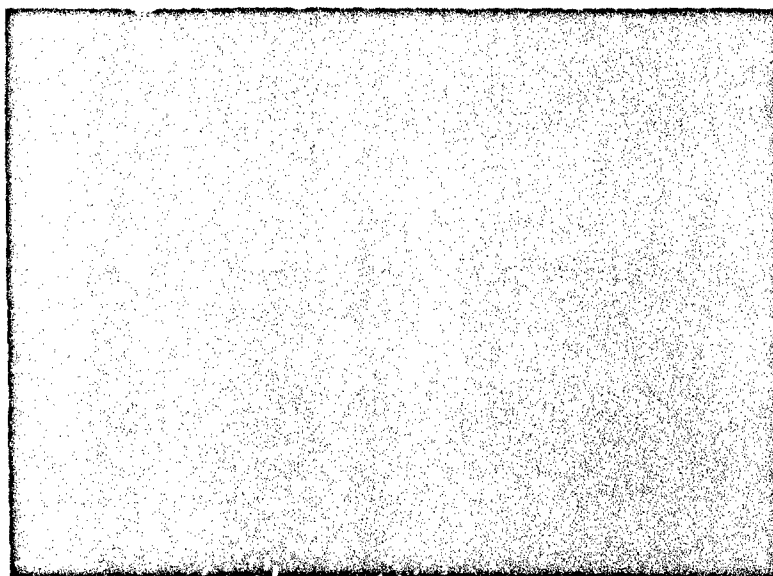


FIGURE 21. 8 DAY PIG NO.7 (ACC 136). MEDIASTINAL LYMPH NODE-NECROSIS, MASSES OF FIBRIN. H & E 200X.

FIGURE 22. 8 DAY DOG NO.25 (ACC 137). LUNG-HEMORRHAGIC & FIBRINOUS PNEUMONIA, COMPLETE OCCLUSION OF BRONCHIOLE & ALVEOLI WITH FIBRIN & CELLS. H & E 175X.

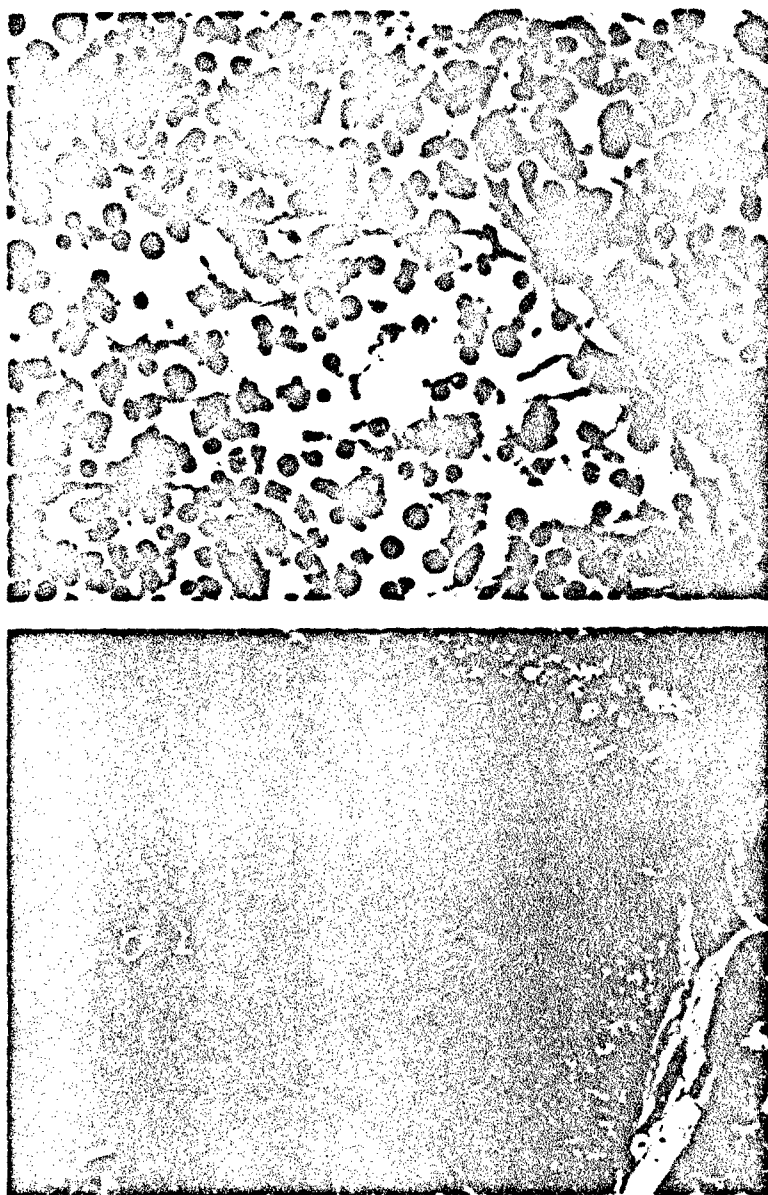


FIGURE 23. 8 DAY DOG NO. 25 (ACC 137). HILAR LYMPH NODE - EDEMA, PLASMA CELLS & MACROPHAGES IN MEDULLARY SINUSOIDS. H & E 900X.

FIGURE 24. 10 DAY PIG NO. 2 (ACC 140). LUNG - HEAVY ACIC & FIBRINOUS PNEUMONIA. H & E 12X.



FIGURE 25. 10 DAY PIG NO 2 (ACC 140). LUNG - HEMORRHAGIC & FIBRINOUS PNEUMONIA. H & E 135X.

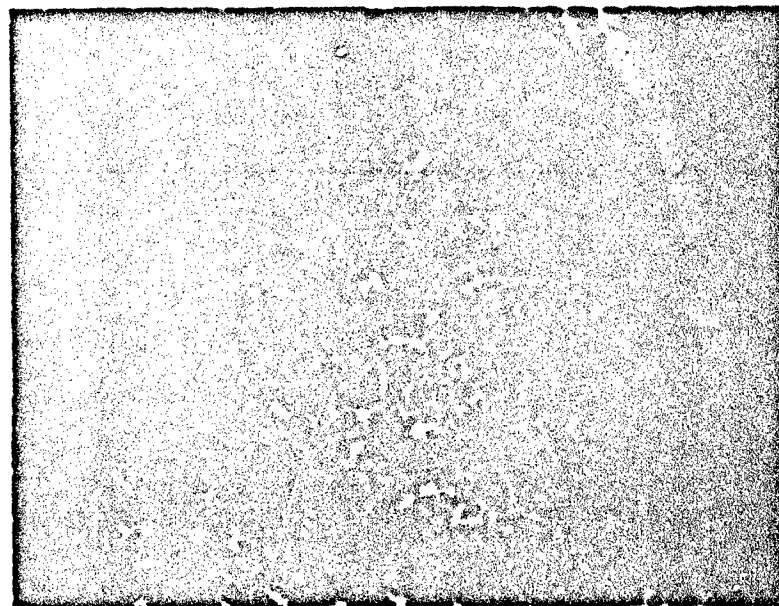


FIGURE 26. 10 DAY PIG NO 2 (ACC 140). HILAR LYMPH NODE - DEPLETION OF MATURE LYMPHOCYTES, LARGE ACTIVE GERMINAL CENTER. H & E 210X.

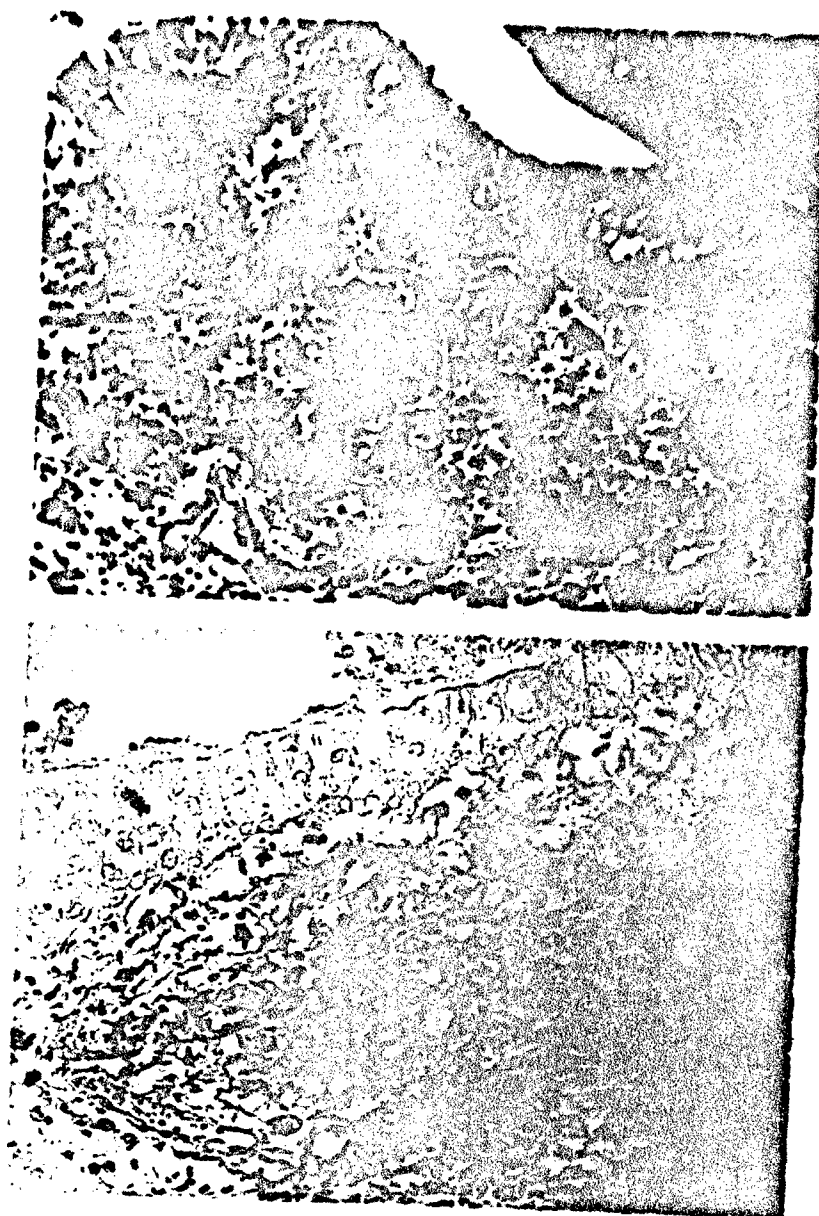


FIGURE 27. 56 HOUR SHEEP NO. 19 (ACC 116). LUNG - LESION IN WALL OF BRONCHIOLE CONTAINING NEUTROPHILS, BACILLI & SOME FIBRIN. H & E 200X.

FIGURE 28. LUNG - SAME AS ABOVE. B & B 400X.

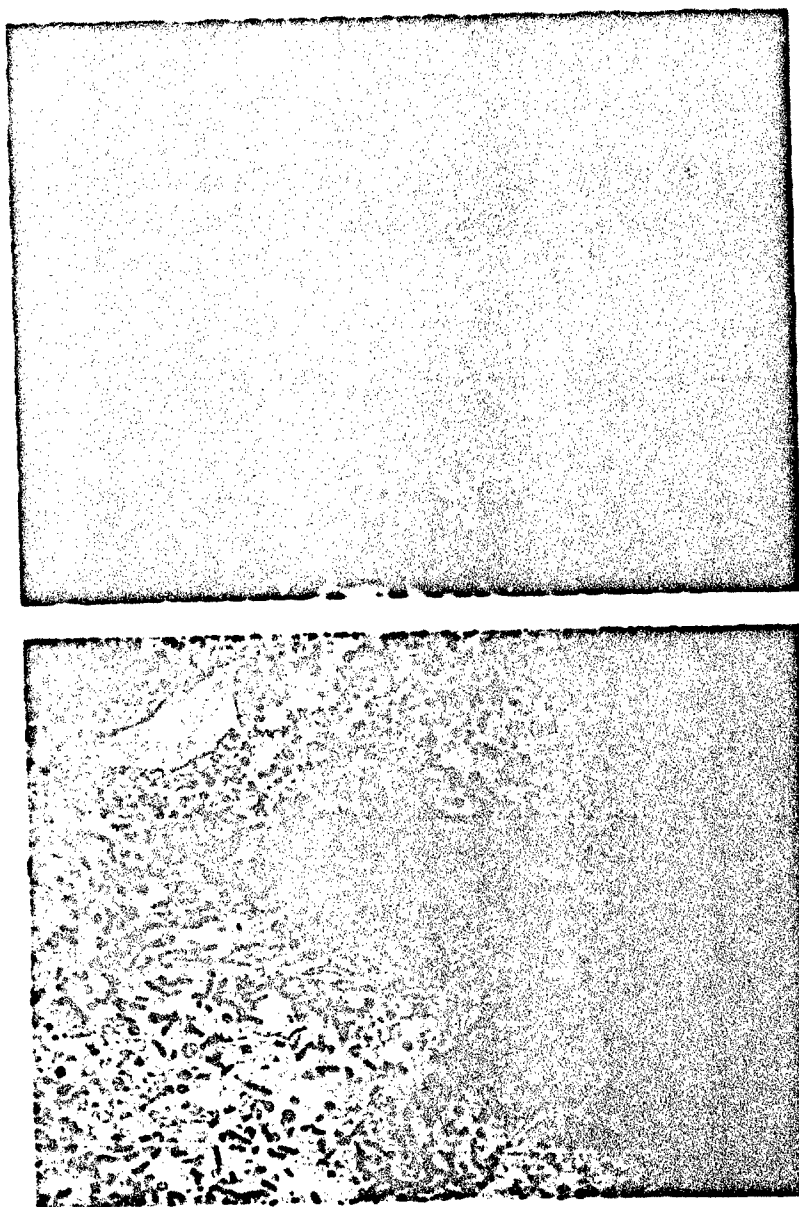


FIGURE 29. 56 HOUR SHEEP NO. 19 (ACC 118). LYMPH NODE - LYSIS OF NODE, MASSES OF BACILLI. H & E 400X.

FIGURE 30. LYMPH NODE - SAME AS ABOVE. B & B 400X.

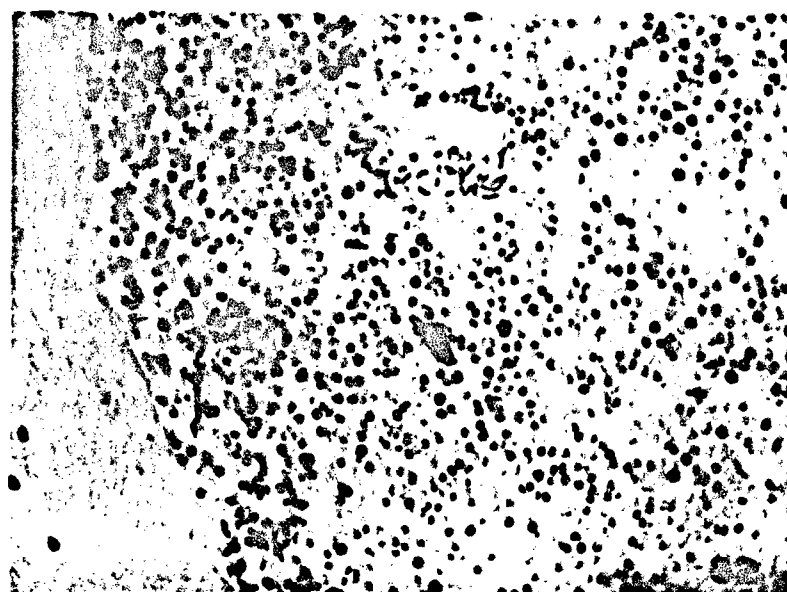


FIGURE 31. 28 HOUR SHEEP NO. 8 (ACC 129). HILAR LYMPH NODE - HISTOLOGIC ARCHITECTURE WITHIN LIMITS OF NORMALCY. H & E 240X.
FIGURE 32. HILAR LYMPH NODE - SAME AS ABOVE. B & B 312X.

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STUDIES ON BACILLUS ANTHRACIS

PART 2

CHEMOPROPHYLAXIS AND CHEMOTHERAPY OF EXPERIMENTAL RESPIRATORY ANTHRAX IN SHEEP

(Gochenour, Gleiser, Gaspar, Overholt, Kuehna, Byron, and Tigertt)

I. INTRODUCTION

Henderson and his associates^{1/} demonstrated the effectiveness of chemotherapy of anthrax in monkeys, using a combination of protective antigen and penicillin. Information was needed on such therapy for sheep, another susceptible species. Previous studies at Dugway Proving Ground (DPG)^{2/} on the pathogenesis of respiratory anthrax in sheep and other animals had indicated that the febrile response was a reliable criterion of infection with Bacillus anthracis. The wind tunnel previously employed in the DPG trials provided a suitable means for simultaneous exposure of a number of animals for such studies.

II. MATERIALS, METHODS, RESULTS AND DISCUSSION

A. GENERAL

The same exposure device and methods were used in these 1958 trials as were employed previously^{2/}. One hundred forty-four sheep weighing 53-73 pounds were procured by DPG; they had been immunized with Pasteurella multocida vaccine. The protective antigen employed had been prepared by Medical Investigation Division, Fort Detrick^{3,4/}, procaine penicillin-G and Tetracycline-Vet (both from Charles Pfizer and Co.) were the antibiotics employed.

Rectal temperatures were obtained twice daily for one week and daily thereafter. Blood cultures were attempted daily or twice daily for five days and at sacrifice or death. At each bleeding approximately 10 ml of blood were drawn; 8 ml were allowed to clot for serological studies at a later time, and 2 ml were inoculated directly into diphasic tryptose medium. The bottles were incubated in the upright position at 37°C and examined at the end of 24, 48 and 72 hours. At each examination any growth was transferred to blood agar and tryptose agar plates. In addition, slides for microscopic examination were made of those colonies morphologically resembling B. anthracis. Following 24 hours' incubation the agar plates were examined and slides made of those isolates not previously examined microscopically. Isolations of the organism were transferred to nutrient agar slants for retention.

Three experiments were conducted; the first on October 10, 1957, was a preliminary run; the second on June 20, 1958 was a prophylaxis trial and the third on July 2, 1958 was a therapy trial. The same respiratory challenge as measured by impinger titrations, was presented on each occasion. In addition one limited trial on therapy was conducted later on subcutaneously-challenged sheep.

B. PRELIMINARY TRIAL

Ten sheep were used in the 1957 trial. Two were controls; four were given 300,000 units of penicillin intramuscularly (IM) every 12 hours for five days and a single dose of anthrax protective antigen, both starting six hours post-exposure (Early Group) and four were given similar drugs and dosages starting 30 hours post-exposure (Late Group).

Figure 1 presents a summary of the temperatures and blood culture findings. (The charts throughout this report do not show absolute temperatures but are plotted as a rounding-off of temperatures of 105.2 to 106.0°F, as 106.0°F, and so on.) It should be noted that in the Early Group, two of four had moderate febrile periods after cessation of drugs. At no time were the animals clinically ill. The Late Group was essentially treated, as these animals became febrile shortly after the initiation of drugs. Drugs controlled fever in three sheep; two were culturally positive at the time of drug initiation. No febrile relapses were noted.

One animal from each therapy group was sacrificed on days 9, 14, 15 and 16. No significant gross changes were observed at autopsy in any of them except No. 5, a 14-day sacrifice animal; this sheep had an enlarged left prescapular lymph node with a necrotic and hemorrhagic center. Histologic examination of this node revealed caseous lymphadenitis. Brown and Brenn (B&B) stain revealed the presence of numerous bacilli morphologically compatible with B. anthracis.

The recovery of anthrax organisms from four of these sacrificed sheep indicated that penicillin did not completely eliminate B. anthracis and that survival through the period of observation was probably attributable to an immune response, and not to a bactericidal effect of drug.

Because of the limited number of controls and the unanticipated survival of one of them, it was not possible to estimate the severity of the presented challenge.

C. PROPHYLAXIS TRIAL

This trial was designed to assess the requirement for use of protective antigen in combination with penicillin or tetracycline in prophylaxis of respiratory anthrax in sheep.

Fifty-eight sheep were used, distributed as follows: Controls--10, Penicillin alone--10, Tetracycline alone--9, Antigen alone--10, Penicillin + Antigen--9, and Tetracycline + Antigen--10. Penicillin and antigen were administered as in the preliminary trial; tetracycline (500 mg per dose) was given on the same time schedule as penicillin. All drugs were started 24 hours post-exposure.

Figures 2 through 7 show temperatures (rounded off), and results of blood culture attempts and IM re-challenges of survivors (21 days post-respiratory exposure). Blood films made on sheep dead or dying were examined microscopically. Autopsies were not routinely performed.

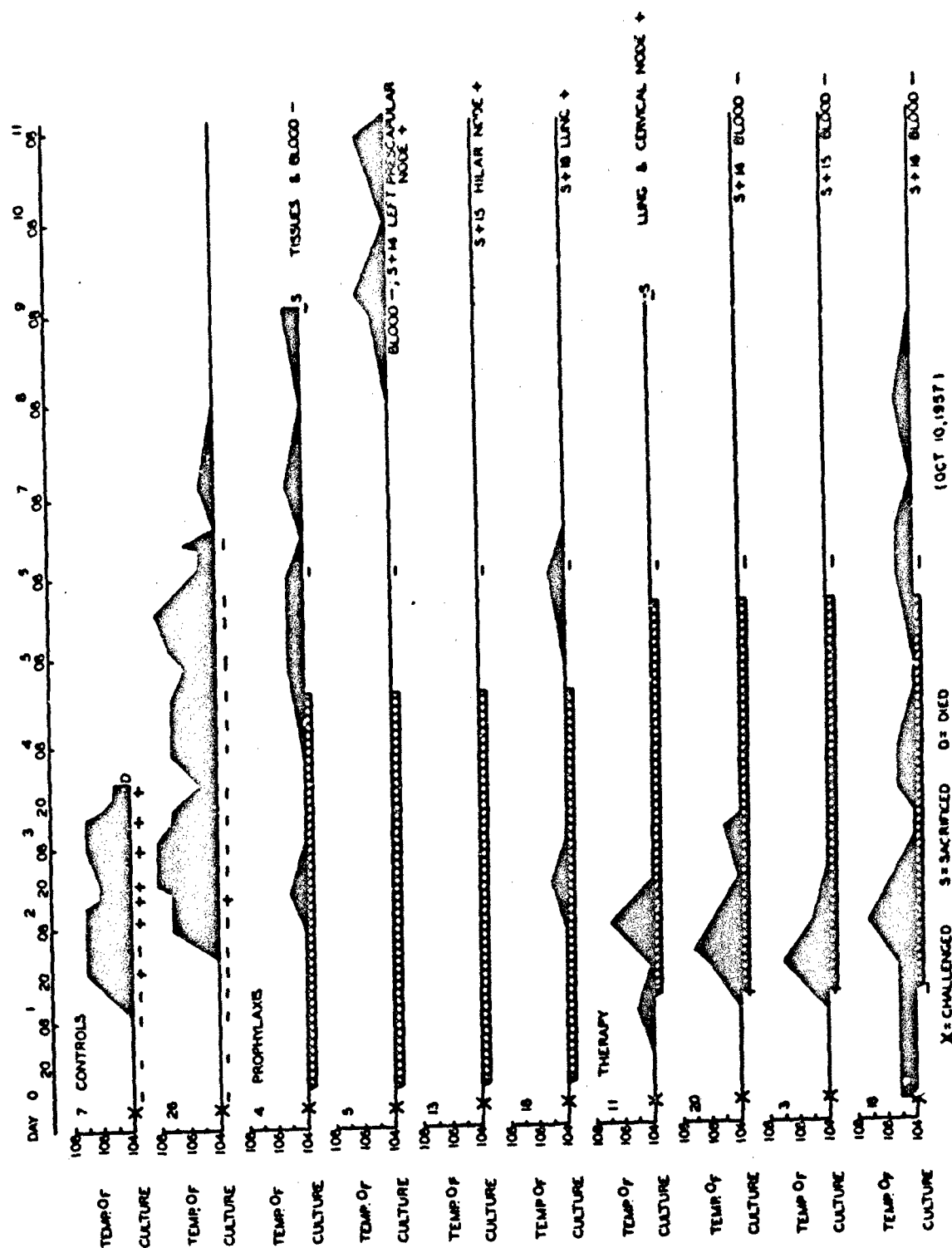


FIGURE 1. TEMPERATURES & CULTURE RESULTS IN CHEMOPROPHYLAXIS & CHEMOTHERAPY SHEEP.
 CHALLENGE ANTIGEN (10 mg) PROCANE PENCILLIN-G (600,000 u DAILY FOR 5 DAYS)



FIGURE 2 TEMPERATURE & BLOOD CULTURE RESULTS IN CONTROL SHEEP

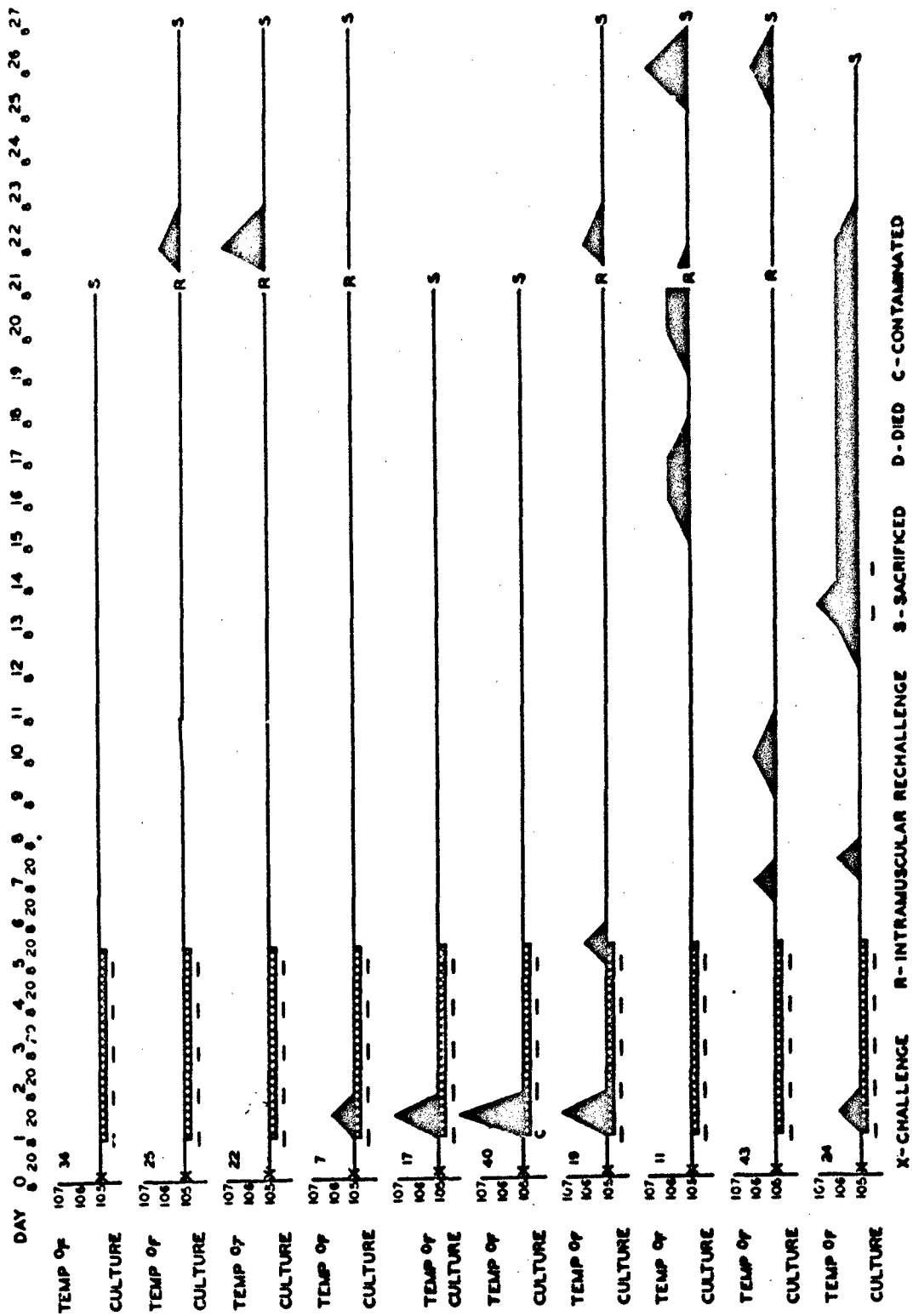
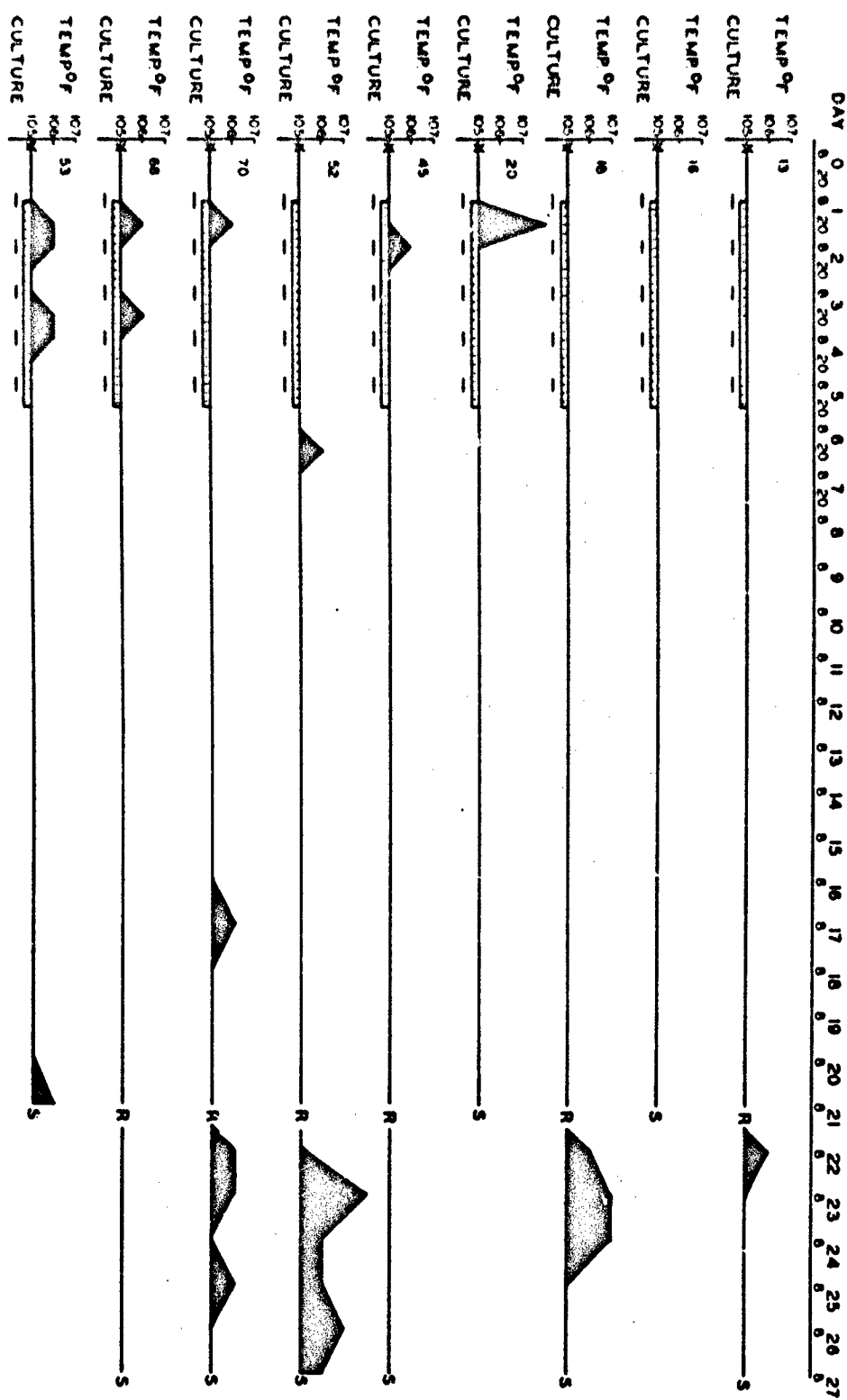


FIGURE 3. SHEEP TREATED WITH PENICILLIN (300,000 u IM bid; TOTAL 3,000,000 u.)



X-CHALLENGE R-INTRAMUSCULAR RECHALLENGE S-SACRIFICED

FIGURE 9. SHEEP INEALCU WITH ICINAWIVLINE (DUU mg 1M DID; TOTAL 5.0 gm.)

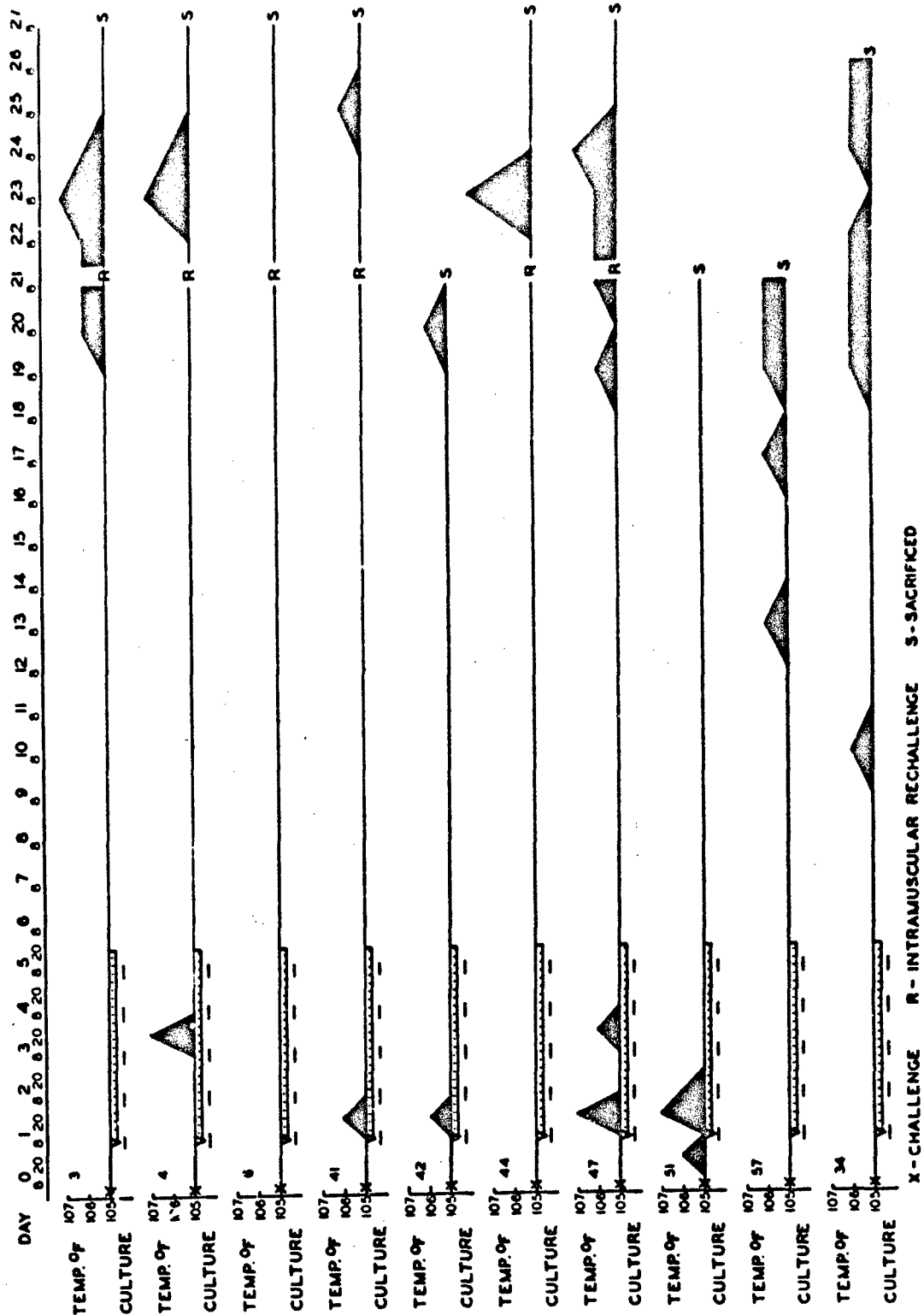


FIGURE 7. SHEEP TREATED WITH ANTHRAX PROTECTIVE ANTIGEN (SINGLE DOSE, 10 ml)
 & TETRACYCLINE (500 mg IM bid, TOTAL 5.0 gm.)

Eight of 10 controls and 6 of 10 sheep receiving antigen alone had positive blood cultures. Penicillin and tetracycline, whether alone or in combination with antigen, apparently prevented the appearance of bacilli in peripheral blood. All sheep that died had demonstrable bacteremias as shown by blood films.

No explanation is available for the failure to infect one control sheep, No. 21, which was shown to be susceptible to anthrax infection when challenged IM 21 days post-respiratory exposure. Uncertainty of the exact challenge dose made interpretation difficult; it is believed that the groups were exposed to approximately one respiratory LD₅₀. The difference in numbers of survivors between control and antigen-alone groups is not considered significant.

The tetracycline caused severe local reactions manifested by tenderness at the site of inoculation and by foot drop and posterior paralysis. With these exceptions all animals receiving antibiotics remained well and showed no clinical signs of illness.

D. THERAPY TRIAL

This experiment was designed to determine whether the drug regimens found satisfactory for prophylaxis of respiratory anthrax were adequate for early chemotherapy.

Fifty-six sheep were employed as follows: Controls--9, Penicillin alone--10, Tetracycline alone--10, Antigen alone--9, Penicillin + Antigen--9, and Tetracycline + Antigen--9. Rectal temperatures were taken every six hours instead of 12. The animals were assigned to test groups and appropriately treated when two successive temperatures of 105.2°F or greater were obtained. Febrile responses of these animals and the results of blood culture attempts are shown in Figures 8 through 13.

Again marked local reactions to tetracycline were seen. There was a fairly satisfactory response to penicillin and tetracycline, with or without associated protective antigen, the temperature usually returning to normal within 24 to 36 hours. However, sheep No. 118 (penicillin group) and No. 130 (penicillin + antigen group) showed much more prolonged fevers, almost throughout the entire period of drug administration. Surviving sheep showed a mild febrile episode between days 10 and 13. A similar, more delayed episode was noted in the prophylaxis animals and was probably a "relapse."

In the penicillin group, sheep No. 120, which died, had been febrile at 48 hours and was placed on therapy eight hours later, at the time of the second positive blood culture. Signs of clinical meningitis appeared; this animal was found dead on the morning of day 6. At autopsy there were two outstanding lesions: severe, generalized, hemorrhagic meningitis (Figure 14) and greatly enlarged, hemorrhagic mediastinal lymph nodes. The spleen was not enlarged.

Microscopic examination of brain confirmed the gross observation of meningitis; the inflammation was acute, and included intense, frank necrosis of brain substance.

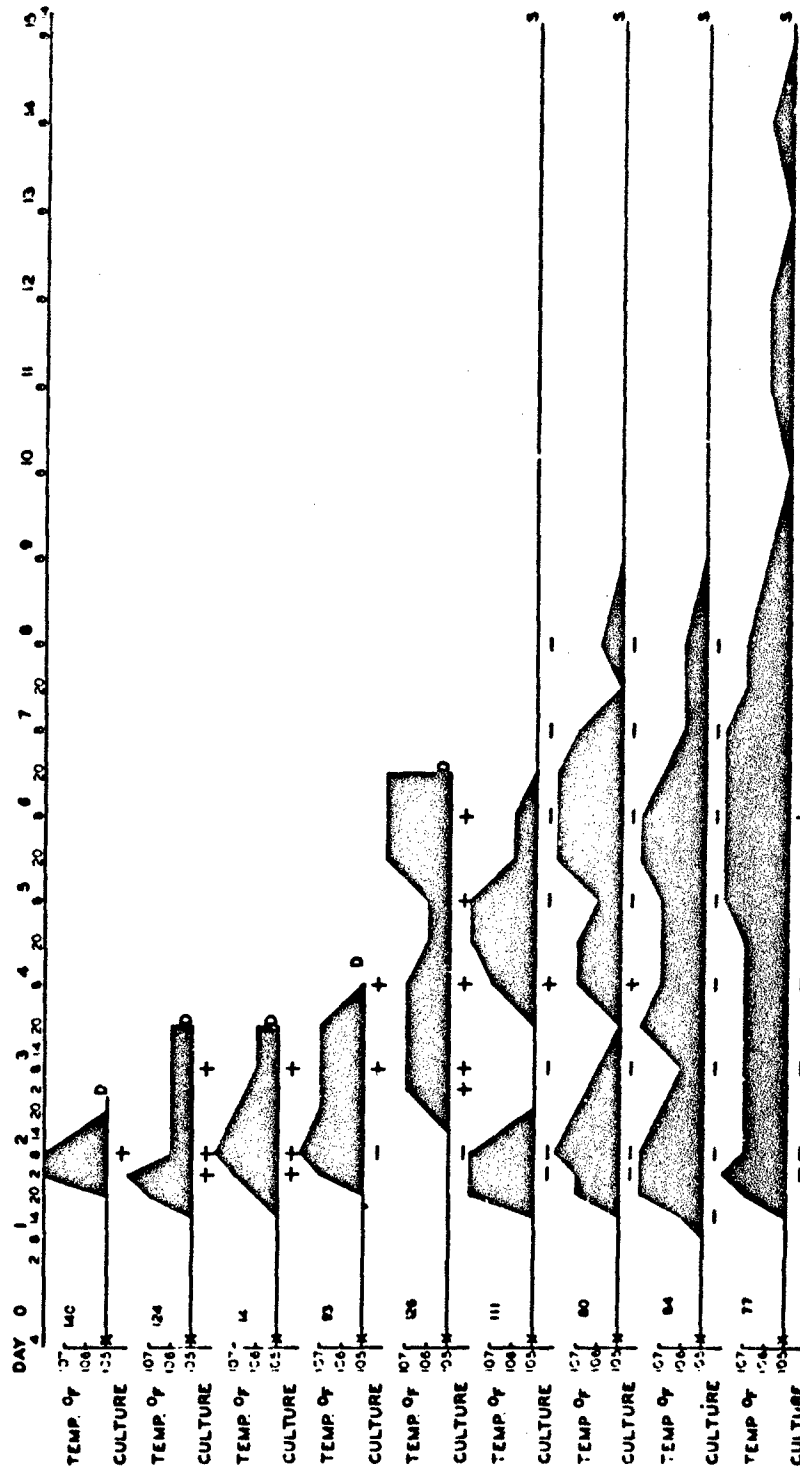


FIGURE 8. CHEMOTHERAPY OF RESPIRATORY ANTHRAX IN SHEEP CONTROL GROUP

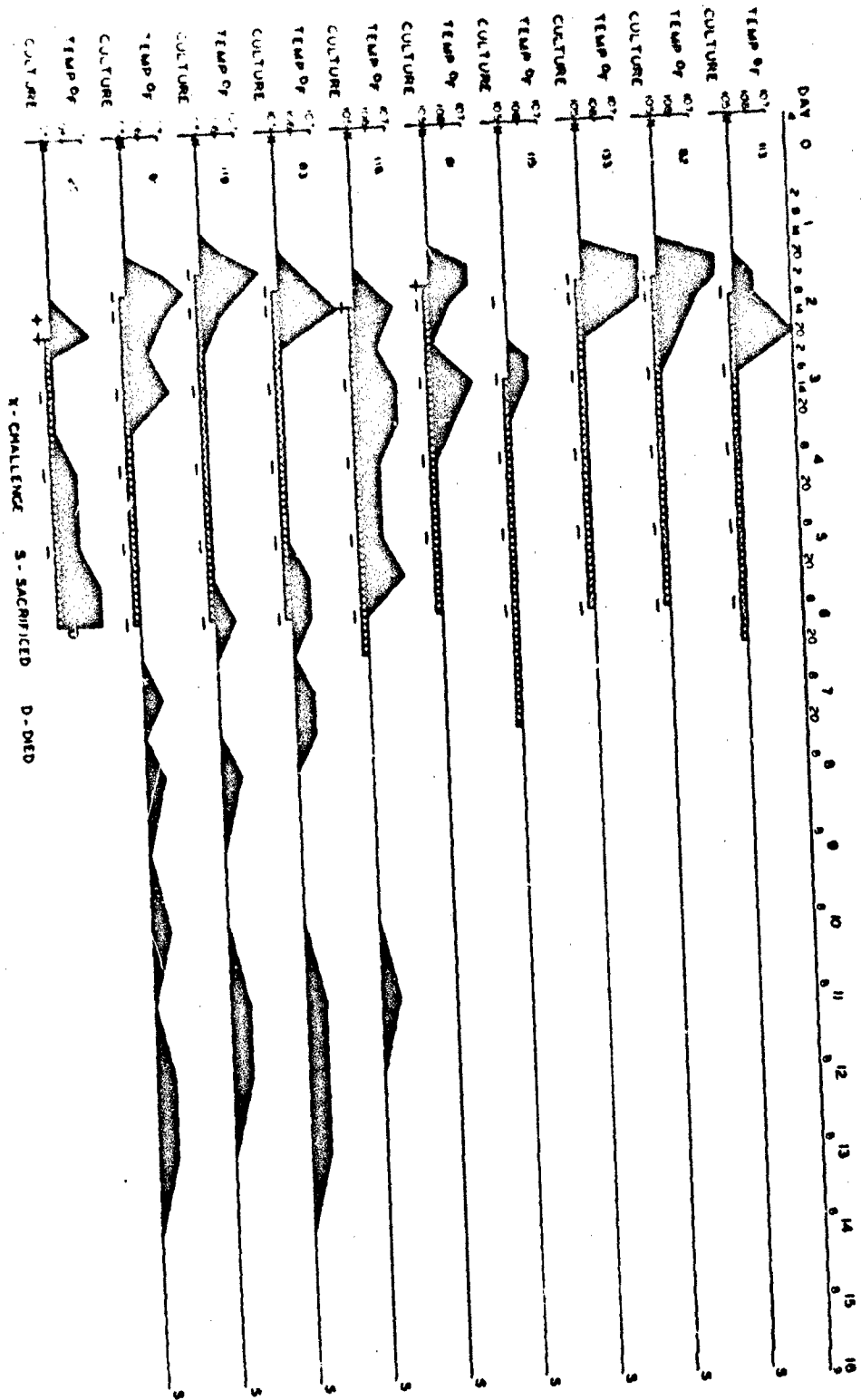


FIGURE 9. SHEEP TREATED WHEN ILL WITH PENICILLIN (300,000 u. 1M bid;
TOTAL 3,000,000 u.)

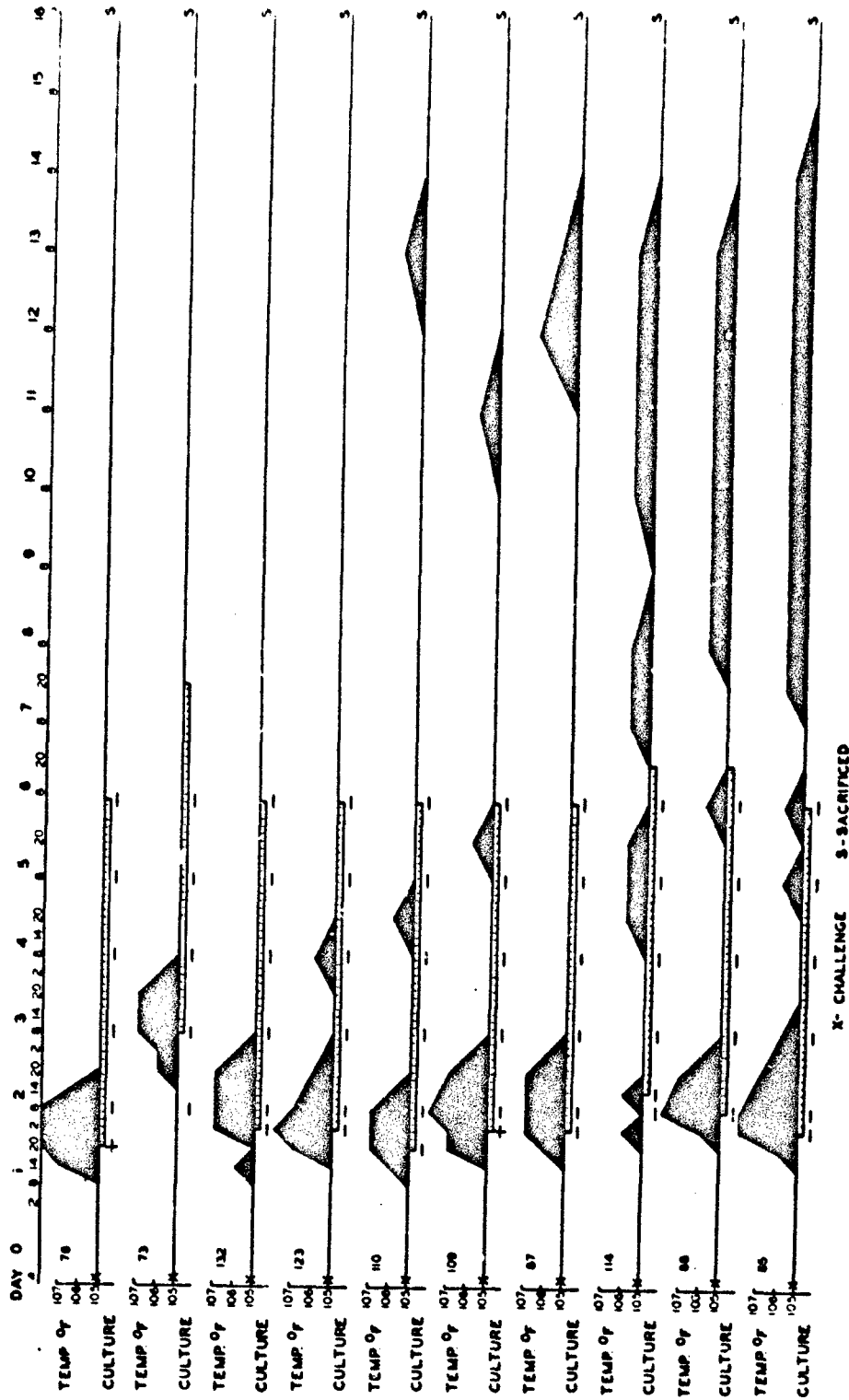


FIGURE 10. SHEEP TREATED WHEN LL WITH TETRACYCLINE (500mg IM bid, TOTAL 50 gm)

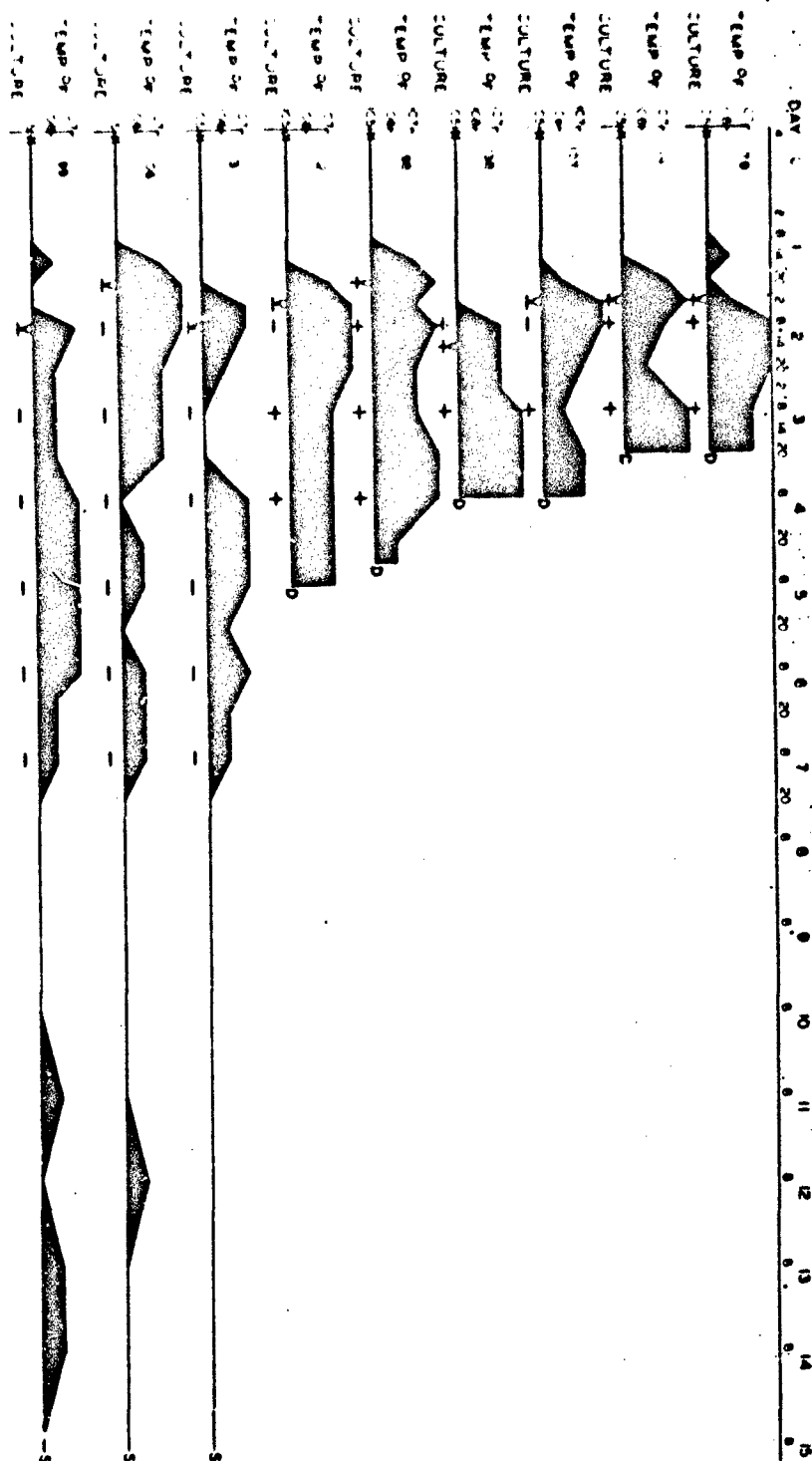


FIGURE 11. SHEEP TREATED WHEN ILL WITH ANTHRAX PROTECTIVE ANTIGEN
(SINGLE DOSE, 1.0 ml)

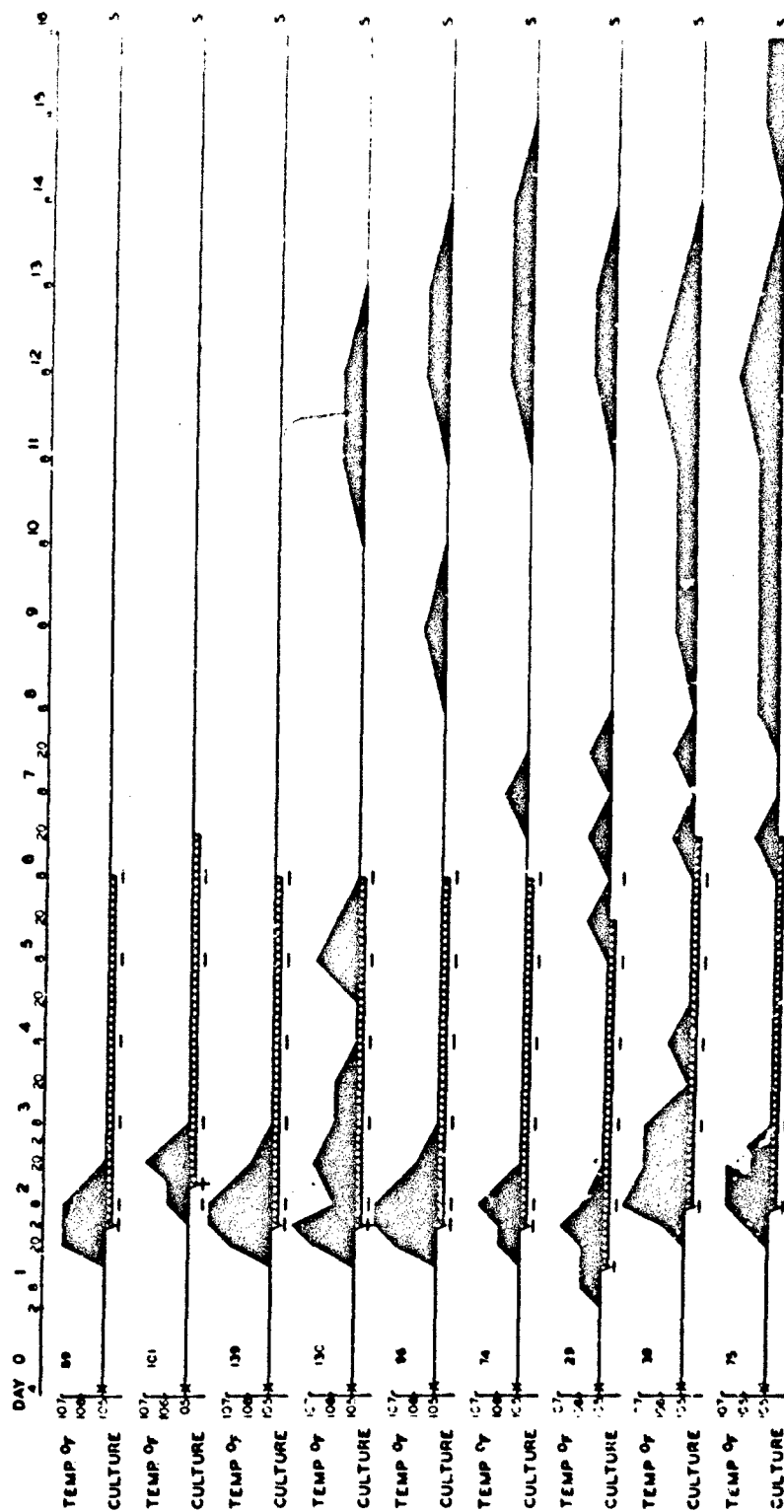


FIGURE 12 SHEEP TREATED WHEN ILL WITH ANTHRAX PROTECTIVE ANTIGEN (SINGLE DOSE, 10ml) & PENICILLIN (300,000 u IM BID, TOTAL 3,000,000 u)

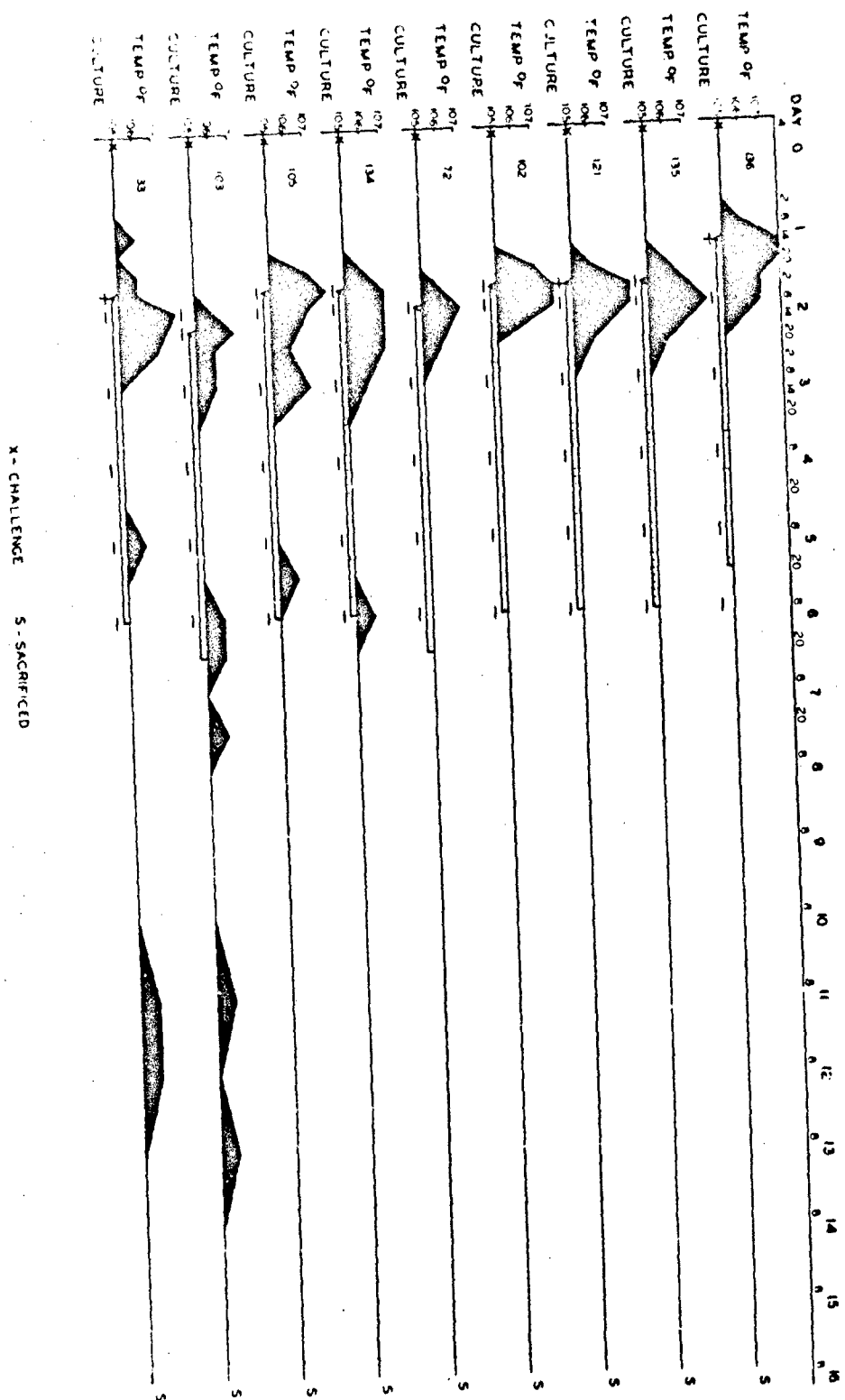


FIGURE 13 SHEEP TREATED WHEN ILL WITH ANTHRAX PROTECTIVE ANTIGEN (SINGLE DOSE, 10 ml) & TETRACYCLINE (500 mg IM bid, TOTAL 5.0 gm)



FIGURE 14 5 DAY SHEEP NO. 120 (ACC 340). HEMORRHAGIC MENINGITIS IN ANIMAL THAT HAD BEEN PLACED ON PENICILLIN THERAPY 6 HOURS AFTER START OF FEVER, SUBSEQUENTLY DEVELOPED CLINICAL MENINGITIS & DIED.

Lungs microscopically showed edema and diffuse hemorrhage into the interstitial structures and pulmonary alveoli. Organisms were recognized within blood vessels. There was some desquamation of the bronchiolar epithelium but this was believed to be due to post mortem degeneration. There was a mild cellular infiltrate around some of the larger bronchioles. Bacilli were seen in several arterioles.

The mediastinal lymph node architecture was completely altered; follicles and medullary cords could not be recognized. Cellular elements were widely separated, suggesting intense edema. Hemorrhage was also present in one section, varying in intensity from area to area. Many neutrophils and blast forms were present. No caseous lymphadenitis was seen.

Focal necrosis of liver parenchyma was found. There was loss of cells in these areas, leaving nondescript, eosinophilic, noncellular material. There was a moderately heavy cellular infiltrate composed of neutrophils and lymphocytes. Many of these cells had undergone degenerative changes. Gram-positive bacilli were recognized by B&B staining in hepatic sinusoids.

Microscopic diagnoses were:

- Meningitis, acute, hemorrhagic, bacterial (anthrax)
- Encephalomalacia and hemorrhage, cerebral & cerebellar cortices
- Necrosis, focal hepatic
- Hemorrhage & edema, diffuse, pulmonary
- Lymphadenitis, acute, hemorrhagic, necrotizing, posterior mediastinal node.

The extent of the mediastinal involvement in this animal was comparable to a 56-hour sacrifice animal previously reported^{2/}; neither animal had caseous lymphadenitis. It is suggested that 4½ days of therapy had merely stabilized the infectious process. Whether the progress of the infection was similar in other animals in the therapy study, and was reversed by treatment, is a problem for further investigation.

In this trial all sheep were infected, since sustained fever post-challenge was the criterion for inclusion in the experiment. Again, the challenge dose appeared to be in the order of one sheep respiratory LD₅₀. No significant difference in morbidity and mortality was evident between the control and antigen-alone groups. Results do not permit unequivocal statements on therapeutic regimen of choice. Based on severity of secondary fever and its incidence, it would appear that tetracycline gave as good results as penicillin, if not slightly better.

E. DELAYED CHEMOTHERAPY OF SUBCUTANEOUS ANTHRAX

A limited trial was conducted to obtain information on the effectiveness of the two antibiotics previously used when started 24 hours after onset of fever in sheep infected subcutaneously.

Twenty sheep, surplus to those required for the respiratory challenges,

were used. In general, they were culls, having been excluded from earlier studies because of severe ovine ecthyma, unexplained fever, or other reasons.

Each animal was challenged subcutaneously with 27,000 spores of B. anthracis. Temperatures were taken every 12 hours; two successive temperatures of 105.2°F or greater was the criterion for assignment to treatment groups. Thirteen sheep received no therapy, four received penicillin and three received tetracycline, both antibiotics being given in the same regimens. Blood cultures were obtained at the time animals were assigned to groups.

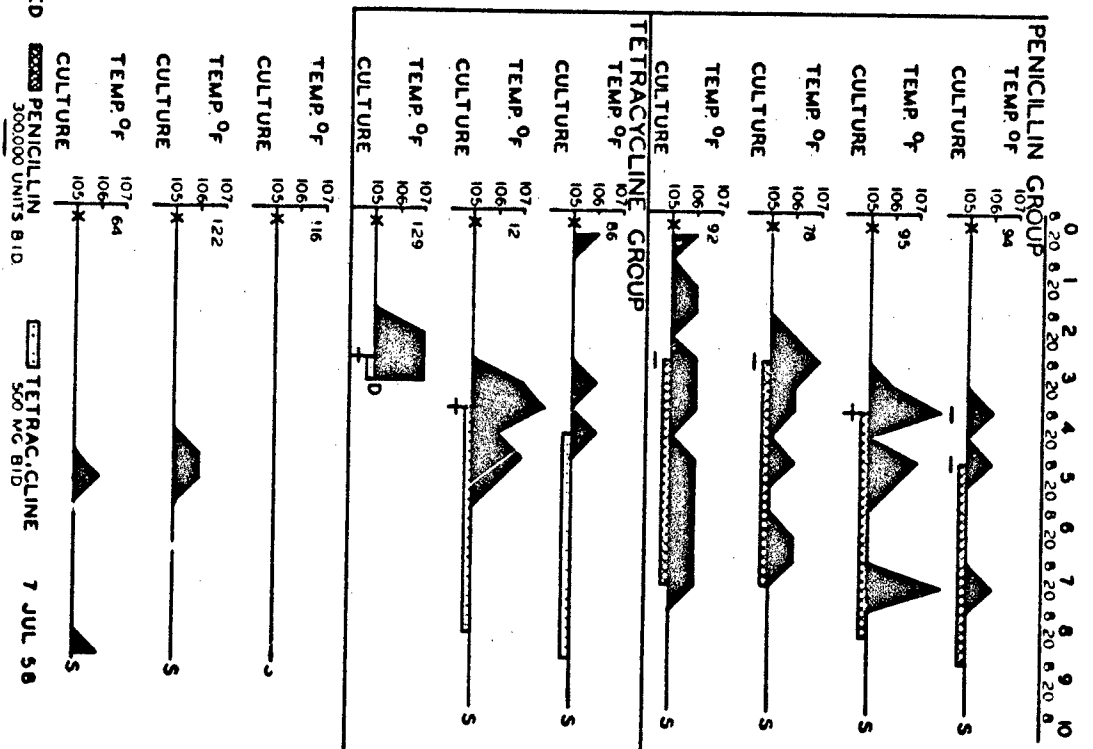
Figure 15 summarizes febrile responses, results of blood cultures and death times. The challenge was lethal to eight controls, six of which had fevers of more than 12 hours duration prior to death. The responses in the five survivors were erratic so that they were never assigned to specific groups and are included with controls. The four penicillin-treated animals survived the period of observation, as did two of three tetracycline-treated sheep.

In summary, it appears that, based on extremely limited information, therapy may be delayed only a relatively short time, probably not more than 24 hours of sustained fever.

III. SUMMARY

Prophylaxis and therapy trials, using penicillin, tetracycline, protective antigen, and combinations thereof were conducted in respiratory-induced anthrax in sheep. Under the conditions of the study, no requirement was found for the use of protective antigen in combination with antibiotic for successful prophylaxis and therapy in contrast with such a requirement in monkeys^{1/}. There was clear-cut evidence that viable anthrax organisms persisted in the sheep after drug was terminated.

A limited therapy trial of penicillin and tetracycline in subcutaneous anthrax in sheep showed that therapy could not be delayed much more than 24 hours.



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STUDIES ON BACILLUS ANTHRACIS

PART 3

PATHOLOGY OF EXPERIMENTAL INTRADERMAL ANTHRAX IN MACACA MULATTA (Berdjis, Gleiser, Hartman and Goehenour)

I. INTRODUCTION

Anthrax has long been recognized as a disease of man and domestic animals but a complete understanding of the disease is still lacking.

The purpose of this paper is to describe the pathology of terminal intradermal anthrax in the unmodified Macaca mulatta and to attempt to elucidate the pathogenesis of this complex disease.

II. MATERIALS AND METHODS

In order to study the histopathology and pathogenesis resulting from intradermal inoculation of Bacillus anthracis in M. mulatta, groups of young immature animals were challenged with 500, 5,000 and 10,000 or more spores of B. anthracis (Table I). A phenolized spore suspension of Vollum-189 was used for these studies. The spores were heat-shocked at 60°C for 30 minutes. The time interval between this procedure and inoculation of the spore suspension never exceeded 7 days and was never less than 24 hours. On the basis of virulence tests in guinea pigs, there was no difference between 1- and 7-day intervals. The spores were suspended in distilled water at 5°C and inoculated intradermally into the forearm. One animal in the high dose group (No. 892) was inoculated subcutaneously. The monkeys were usually placed in primate chairs. Continuous temperature readings were made by rectal thermocouples during the entire period of the experiment. Blood cultures and physical examination, including inspection of the site of inoculation, blood pressure determinations and EKG evaluations, were performed daily. Blood clotting time, prothrombin time, leukocyte count, and differentials, and bacteriological estimates were completed in some animals. Titrations for guinea pig virulence were made frequently, employing 1-, 10- and 100-cell intradermal challenges. The spores used for titration were at the same suspension, same intervals and same conditions as those used for monkey inoculation. Slurry suspension in 0.1-ml amounts, containing 1, 10 or 100 spores, were inoculated subcutaneously into groups of 6 guinea pigs. All 6 guinea pigs died at the 100-spore challenge level and 5 or 6, of 6, at the 10-spore level. The LD₅₀ is considered to be in the range of 1 to 4 spores.

All monkeys were allowed to die, and autopsies were performed immediately after death. Blood smears and spleen impressions were checked at the time of autopsy for the presence of bacilli. Blood cultures were performed at death if organisms were not noted by direct examination of blood films. The tissues were fixed in 10 per cent neutral formalin, embedded in paraffin, sectioned at 6 μ and stained with routine hematoxylin and eosin (H&E) unless special mention is made. Brown and Brenn staining procedure (B&B) for demonstrating microorganisms in tissue sections was employed. Periodic Acid-Schiff reaction (PAS),

counterstained with light green or hematoxylin was used for evaluating PAS positive material in the hepatic cells.

III. RESULTS

A. CLINICAL OBSERVATIONS

Detailed clinical data and laboratory investigations will be reported elsewhere. A brief summary of these observations, however, is warranted. The clinical response of the unmodified M. mulatta to a challenge of B. anthracis spores given intradermally, or subcutaneously in one monkey, was found to be inconsistent; the essential features may be stated as follows: there was no consistent febrile response; some monkeys developed fever, others did not (Table I). Local reactions at the site of inoculation likewise varied in intensity; in some instances, there was massive cellulitis, involving the forearm and occasionally the whole arm (Figure 1), in others, the site was erythematous and raised. Frequently it was soft and fluctuant, or occasionally ulcerated or erythematopapular. In still others, the animals developed small circumscribed, raised papular, erythematous lesions which did not progress into diffuse cellulitis, and which sometimes regressed before the death of the animal. Shock with respiratory distress and a precipitous drop in blood pressure just before expiration was observed several times.

Laboratory data which included urinalysis, routine hematologic studies and blood clotting studies were not particularly fruitful except that there was no evidence of renal failure or shutdown prior to death.

Blood cultures of the monkeys receiving 5,000 spores or more were frequently positive on day 2 post-challenge, and on day 1 for 500-spore animals (Table I). In 500-spore animals the bacteremia ended on day 2 or 3 only to reappear shortly before death. In 5,000-spore animals the blood cultures often remained positive until death. Blood smears at time of death always contained bacilli (Table I).

B. GROSS PATHOLOGY

Skin and Subcutaneous Tissues - Site of Inoculation: (Figure 1) Cellulitis of the forearm and arm was one of the most conspicuous findings. This lesion grossly appeared as a thick, glistening, moist gelatinous edema infiltrating to a varying degree the subcutaneous tissues; at times it extended into the muscle bundles. This lesion was frequently hemorrhagic, and partly necrotic. The exposed and cut surfaces might show the subcutaneous tissue of the entire arm as moist, shiny and glistening with diffuse edema sometimes extending into the axillae. The connective tissues surrounding the corresponding axillary lymph nodes were also edematous and glistening. The central portion of the lesions were frequently reddish-purple or hemorrhagic. Varying amounts of edema fluid or bloody fluid oozed from cut sections.

In those cases in which the lesions had regressed prior to death, the site of inoculation was firm and showed a discrete or relatively small area of induration and occasional hemorrhage.

TABLE I. CLINICAL AND LABORATORY DATA FOR MONKEYS WITH EXPERIMENTAL INTRADERMAL^{a/} ANTHRAX

DOSE (Spores)	MONKEY WEIGHT NUMBER AT DEATH (Acc. No.) (kg)	MAXIMUM TEMPERATURE (°F) (Day)	CLINICAL SIGNS OF LOCAL REACTION	DEGREE BLOOD CULTURE ^{b/} OF LOCAL (Days)		RESULTS Negative		BLOOD SMEAR RESULTS Before Death At Death Of		DAY OF DEATH
				Positive	Negative	(Days)	(Days)	(Days)	(Days)	
500	809 (642)	4.2	103.8 (9)	Respiratory distress	+++	1,3,16, 17	2,4,8,9, 10	+	+	17
	810 (634)	3.8	105.9 (9)	Coma, shock	+++	1,2,3,9	8	+	+	10
	812 (631)	4.6	100.0	NRE/	+++	0,1,2,8	3,4	NDd/	+	8
	C-9 (662)	3.3	100.0	NR	+	2,3	1	ND	ND	3
5,000	723 (644)	2.5	103.0 (4)	Depression	++	2,3,4,5	0,1	+	+	5
	743 (671)	3.6	105.0 (3)	Depression, gasping	++	2 (3,4 con- taminated)	1	ND	-	5
	705 (643)	2.4	103.2 (4)	Respiratory distress	+	3,4,5	1,2	ND	+	5
	766 (670)	2.3	104.4 (3)	Respiratory distress	+++	2,3	1	ND	+	3
10,000	892 (899)	3.0	104.2 (4)	Cyanosis, vomiting	+++	4,5	1,2,3	ND	+	5
40,000	678 (814)	2.3	104.4 (2)	Cyanosis (moribund)	+++	2,3,4	1	ND	+	4

a. Intradermal except Monkey No. 892 - subcutaneous.

b. Days not recorded, blood culture was not done.

c. NR = Not remarkable.

d. ND = Not done.

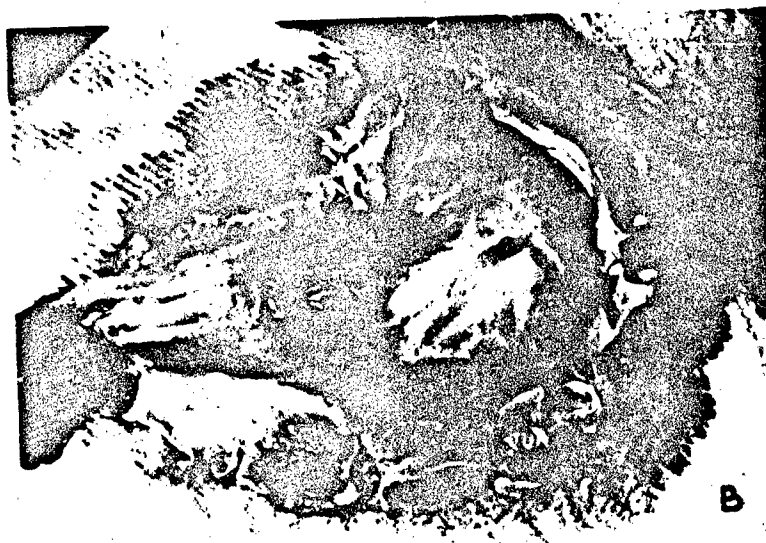
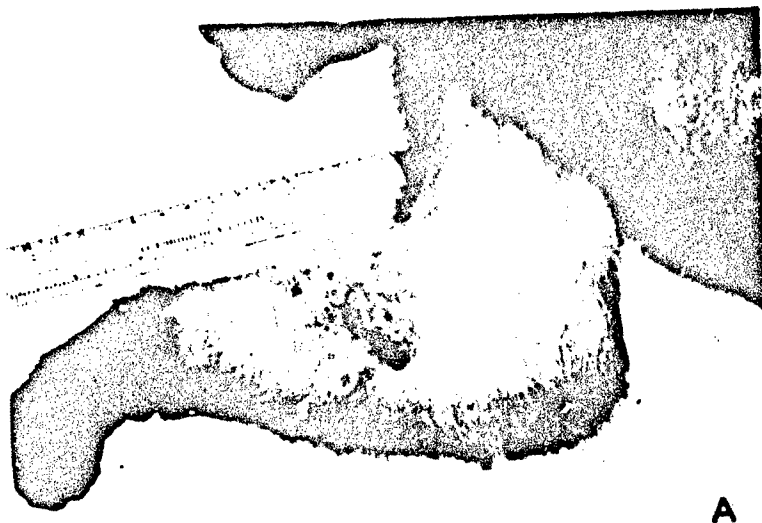


FIGURE 1. SITE OF INOCULATION, ARM & FOREARM (ACC. 642, 500-
SPORE GROUP) (A) PAPULOERYTHEMATOUS LESION WITH
MARKED SWELLING. (B) SAME, AFTER DISSECTION OF SKIN.
SHINY, DIFFUSE, GELATINOUS EDEMA, SOME HEMORRHAGE
OF SOFT TISSUES.

Lymphatic System: The tributary axillary lymph nodes were frequently enlarged, soft and moist. They were surrounded by gelatinous or hemorrhagic edema; the capsular surfaces were wet, shiny, and hemorrhagic. These lymph nodes were often congested and edematous, and, in cross section revealed small or massive hemorrhages sometimes associated with necrosis. The axillary lymphadenopathy sometimes extended to the other lymph nodes and sometimes was generalized. In a few cases the lymphatic system appeared unchanged.

The tonsils were generally inconspicuous.

Spleen was either of normal size or enlarged. The capsular surface occasionally had a bluish tinge and somewhat rounded edges. The consistency was frequently soft. Blood exuded freely from the cut surfaces. The lymphoid follicles were sometimes obscured.

Respiratory System: Although blood-tinged, frothy fluid was present in the larynx of some animals, no significant lesions were grossly detectable in the upper respiratory tract. The tracheobronchial mucosa was frequently injected. Disseminated petechial hemorrhages or focal small hemorrhages, with or without pulmonary congestion or occasional partial consolidation of the lungs, were sometimes visible. Pulmonary edema and hemorrhage of varying intensity were occasionally seen. Lung mite lesions were frequently observed. These lesions, discrete, prominent, isolated or multiple and often subpleural, were sometimes accompanied by small congested areas appearing as red halos. They are described more fully in Part 3^{1/2}.

A minimal amount of mediastinal edema was occasionally present when the mediastinal lymph nodes, including hilar and tracheobronchial, were affected. Prominent edema of the mediastinum was observed in only one monkey (No. 644).

Cavity Fluid: In five instances varying amounts of ascites were observed, twice accompanied by hydrothorax and once by hydropericardium (Table II). Association of pleural, pericardial, and peritoneal effusion was encountered in one animal only (No. 644).

Gastrointestinal Tract: Lesions of oesophagostomiasis were observed in a large percentage of the animals in the mesentery and in the walls of the large intestine. Specific gross lesions that could be attributed to anthrax were not recognized.

Endocrine Glands: Isolated or multiple small foci of hemorrhages were occasionally observed in the adrenal cortices. No other lesions were detected in the endocrine organs.

Central Nervous System: One case of hemorrhagic meningitis associated with mediastinitis and hemorrhagic lymphadenitis, involving the hilar and tracheobronchial lymph nodes was observed. Otherwise the central nervous system was spared.

TABLE II. GROSS PATHOLOGY IN MONKEYS WITH EXPERIMENTAL INTRADERMAL^a/ ANTHRAX

DOSE	MONKEY NO. (Spores)	LYMPHATIC SYSTEM ^b / (Acc. No.) Lymph Nodes	Spleen	MEDIASTINUM	RESPIRATORY SYSTEM	CAVITY FLUID	LIVER	ADRENAL	CNS
500	809 (642)	Congestion, Edema			Hemorrhages, multiple, Edema				
	810 (634)	Hemorrhage, Congestion Edema			Petechial hemorrhages		Congestion Hemorrhage, focal		
	812 (631)						Mottling, Hemorrhage, Congestion focal		
	C-9C/ (662)	Hemorrhage, Enlarged, Edema			Hemorrhage, Ascites massive, Edema		Congestion		
5,000	723 (644)	Hemorrhage, Edema		Mediastl- nitis, mild	Hemorrhage, Ascites, Hydrothorax, Hydropert- cardium		Congestion		Hemor- rhagic meningitis
	743 (671)				Ascites, Hydrothorax		Congestion		
	705 (643)	Congestion, Congestion Edema, Hemorrhage, focal			Hemorrhage, Ascites massive, Edema		Congestion Congestion		Edema
	766 (670)	Congestion, Enlarged, Edema		Mediastl- nitis, severe				Cortical hemorrhage	
10,000	892 (899)	Congestion, Enlarged, Edema			Petechial hemorrhages		Congestion Edema, Congestion		Edema
	678d/ (814)	Hemorrhage, Edema				Ascites		Cortical hemorrhage	
40,000									

- a. Intradermal except Monkey No. 892 - subcutaneous.
b. For cellulitis at the site of inoculation and the day of death see Table I.
c. Kidney was congested.
d. Hemorrhage of colon.

Skeletal Muscle: Extension of the massive gelatinous edema of the skin and subcutaneous tissues at the site of inoculation of those cases that developed an intense cellulitis, into and between the skeletal muscle bundles, was the only evidence of involvement of the striated muscles.

Genitourinary and Cardiovascular Systems, Salivary and Thymus Glands, and Bone and Bone Marrow: No gross lesions were observed.

C. MICROSCOPIC EXAMINATION

Skin and Subcutaneous Tissues: The skin and subcutaneous tissues at the site of inoculation and at times of the entire forearm and arm were altered to varying degrees by a cellulitis characterized by massive edema, inflammatory infiltration, hemorrhage and necrosis.

The surface epithelium was generally little affected. There was, at times, minimal to marked edema in the corium accompanied by a few scattered inflammatory cells especially about the blood vessels at the dermal-epidermal junction. Below this level the tissues were markedly edematous and contained cellular infiltrates and areas of frank necrosis (Figure 2). The inflammatory infiltrates, chiefly leukocytes, with a large number of eosinophils and monocytes, were numerous; they were diffusely distributed or in foci about the blood vessels. A large number of bacilli were evenly distributed throughout the affected tissues in this area. Diffuse hemorrhage and necrosis were also a part of these phlegmonous lesions. The blood vessels, particularly the arteries, contained bacilli and inflammatory cells and in some cases showed minimal to marked necrotizing changes. Widespread inflammatory infiltrates were especially marked about these vessels and around the necrotic areas. Some inflammatory cells, necrosis and hemorrhage were occasionally present about the cutaneous appendages. The adjacent skeletal muscle frequently was damaged, revealing a similar picture. A large number of inflammatory cells were visible between the muscle bundles.

Lymphatic System; Lymph Nodes: The reaction of the lymphatic tissues was variable between animals and within the same animal. Principal histologic features were necrosis with marked depopulation of the lymphoid elements, hemorrhage, inflammatory infiltration of varying intensity, and varying numbers of bacilli. Cellular infiltrations were made up of neutrophils and monocytes. The monocytes frequently had phagocytized erythrocytes, bacilli, and cellular debris (Figure 3).

In some nodes the general architectural pattern was well preserved, in others, those severely involved, the pattern was in complete disorder.

In the lymph nodes with minimal to mild injury the histological picture was essentially that of some necrosis of lymphocytes with the resulting cellular debris in the dilated sinuses and in the follicles, a small number of neutrophils, some edema and congestion, and some bacilli, as individual organisms or in pockets. In the severely affected nodes, there was marked necrosis, with severe depopulation, which give the impression of a "lysing necrosis." Hemorrhage and masses of bacilli were frequently associated with this necrosis.

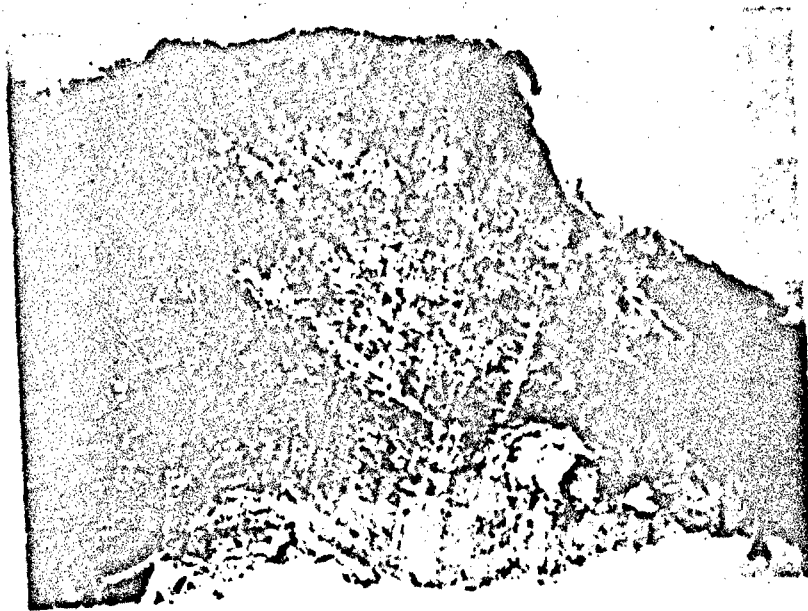


FIGURE 2. SITE OF INOCULATION. (ACC. 642, 500-SPORE GROUP)
EXTENSIVE PHLEGMONOUS CELLULITIS, WITH DIFFUSE
EDEMA, MARKED NECROSIS & DISSEMINATED INFLAMMATORY
INFILTRATES. H & E (A) X9 (B) X80.

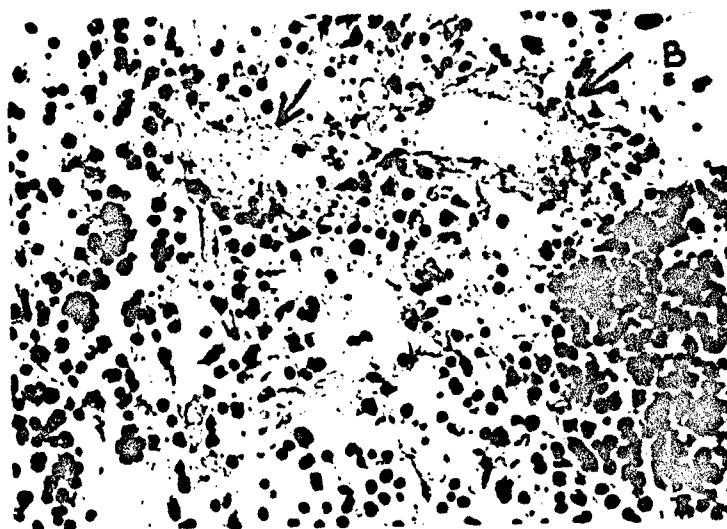
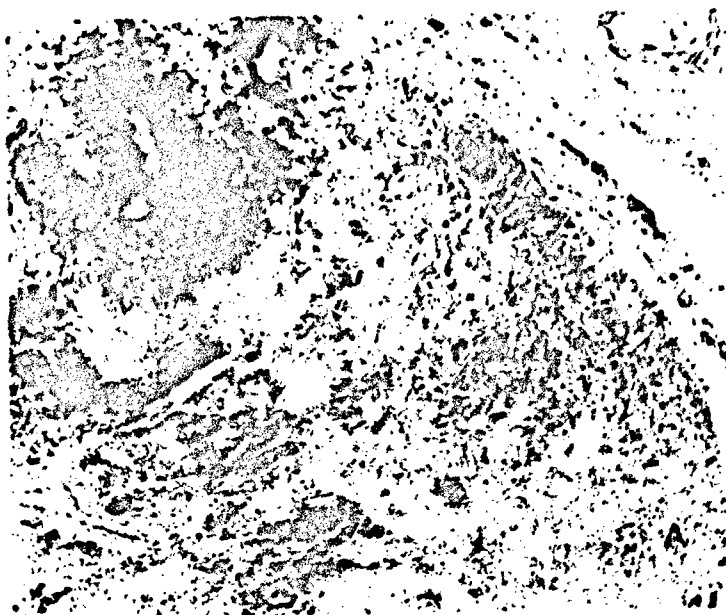


FIGURE 3. LYMPH NODE (ACC. 844, 5000-SPORE GROUP).
NECROTIZING, HEMORRHAGIC LYMPHADENITIS WITH MARKED
TISSUE DESTRUCTION & INFLAMMATORY REACTION.
NECROTIZING VASCULITIS SHOWN IN (B). H&E (A) X35. (B) X350

Mononuclear phagocytes frequently containing cellular debris were very prominent. Erythrophagocytosis was also observed.

The lymph nodes were generally hyperemic with some proliferation of the reticulum cells regardless of the degree of injury. In some nodes this proliferation of reticulum cells was quite prominent.

Hemorrhage was prominent in some nodes, minimal in others. Sometimes, the hemorrhage was seen in association with the necrosis, at other times one occurred without the other. The degree of necrosis appeared to be related to the numbers of bacilli present. However, nodes containing small numbers of bacilli, particularly in the vessels, with minimal other changes were observed.

Spleen: The spleen was frequently the site of extensive and severe changes. There was dilatation and engorgement of the sinuses, with necrosis of cells in follicles and pulp (Figure 4). The degree varied from one animal to another and from one area to another in the same spleen. Accumulations of leukocytes, especially mononuclear cells were frequently seen in the pulp and sinuses, and occasionally in the follicles. Plasma cells and varying numbers of phagocytizing monocytes containing erythrocytes, bacilli or cellular debris commonly occurred. Bacilli were prominent within the splenic parenchyma. Necrosis and hemorrhage were often present not only within the red pulp but also at the level of the follicles, some being entirely destroyed and replaced by hemorrhage with or without necrosis. The picture of the severely damaged spleen was that of severe depopulation (disruption of the architectural pattern), hemorrhage, necrosis, and tremendous numbers of bacilli.

In the less affected spleen there were at times minute or annular hemorrhages at the peripheral portion of the follicles. Usually the Malpighian corpuscles were partially atrophic and often had prominent germinal centers. In occasional cases, there was necrosis of isolated vessel walls with prominent or moderate perivascular inflammatory reaction.

Upper Respiratory Tract: Minimal edema with varying degrees of submucosal congestion were observed in the upper respiratory tract of some animals. No lesions of the epithelial lining of the mucosa were recognized.

Lungs: Lesions of pulmonary acariasis were seen in lungs of practically all animals. A detailed description is given in another section on respiratory anthrax where they seemed to play a significant role¹. These parasitic lesions in the present series of animals were not modified by the infection, except in one case (No. 892) in which there were masses of bacilli present (Figure 5).

The pulmonary changes that were seen were essentially those of septicemia and bacteremia. Edema and hemorrhage of varying intensity were observed several times. In two instances the hemorrhage was considered massive (No. C-9 and 705, Tables II and III). Diffuse inflammatory infiltrates, consisting chiefly of monocytes, were observed primarily in the interstitial spaces and in the alveolar walls (Figure 6). In some animals the lung parenchyma contained myriads of bacilli. These bacilli were primarily in the interalveolar capillary spaces, and appeared to literally "bust out" of the capillaries; in other cases, only a few, or no, bacilli were observed.

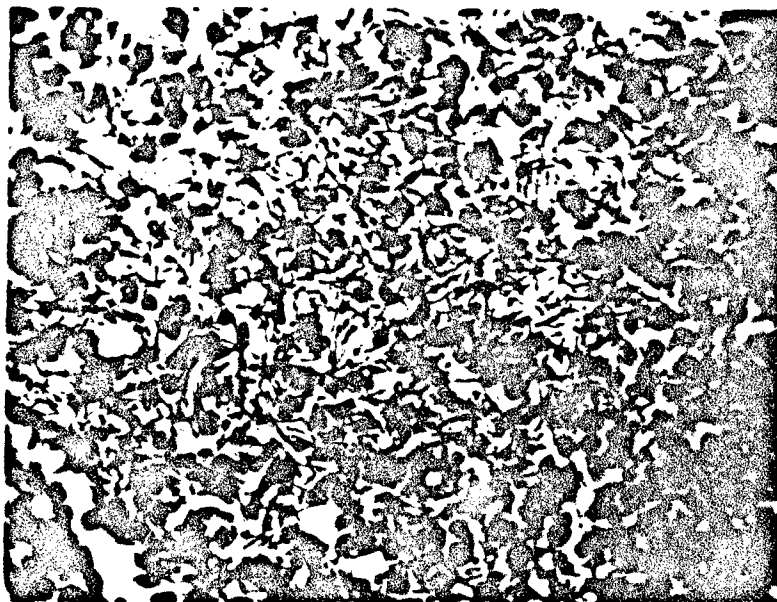
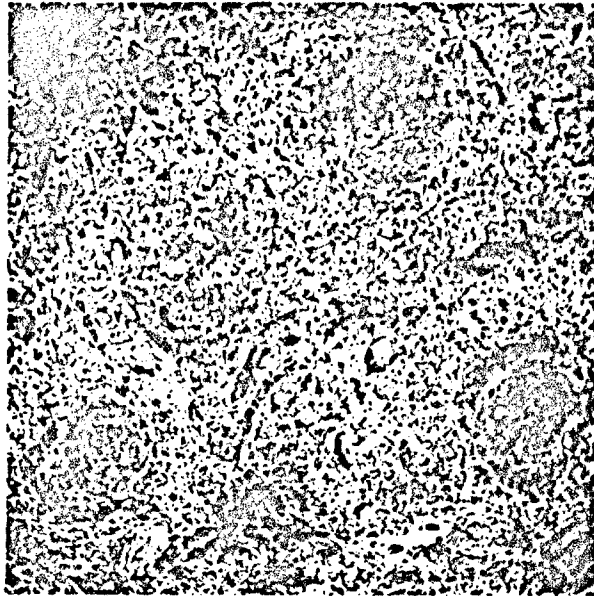


FIGURE 4. SPLEEN. SEPTIC SPLENITIS, WITH HEMORRHAGE & ATROPHY OF FOLLICLES, FOCAL OR DIFFUSE NECROSIS & MYRIADS OF BACILLI EVENLY DISTRIBUTED THROUGHOUT. H & E (A) (ACC. 834, 500-SPORE GROUP, X57) (B) (ACC. 814, 40,000-SPORE) X385.



FIGURE 5. LUNG (ACC. 899, 10,000-SPORE) (A) LUNG MITE LESION WITH SEVERE INFLAMMATORY REACTION (BRONCHIOLITIS & PERIBRONCHIOLITIS). H & E X22, (B) PORTION OF BRONCHIOLAR WALL SHOWING NUMEROUS BACILLI, B & B, X302.

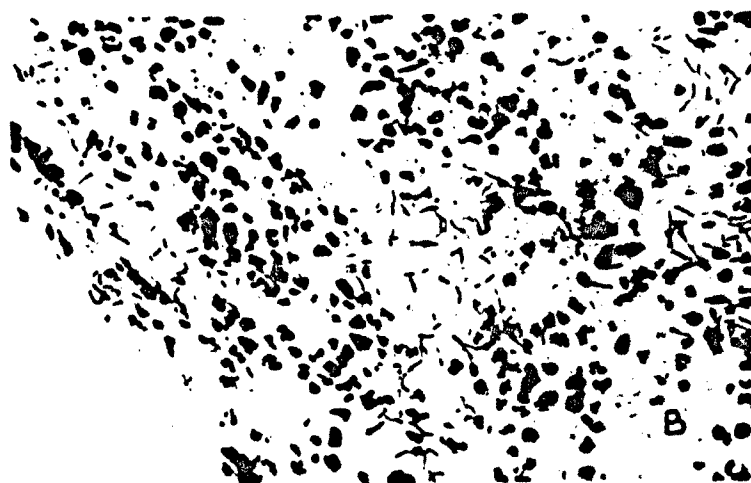
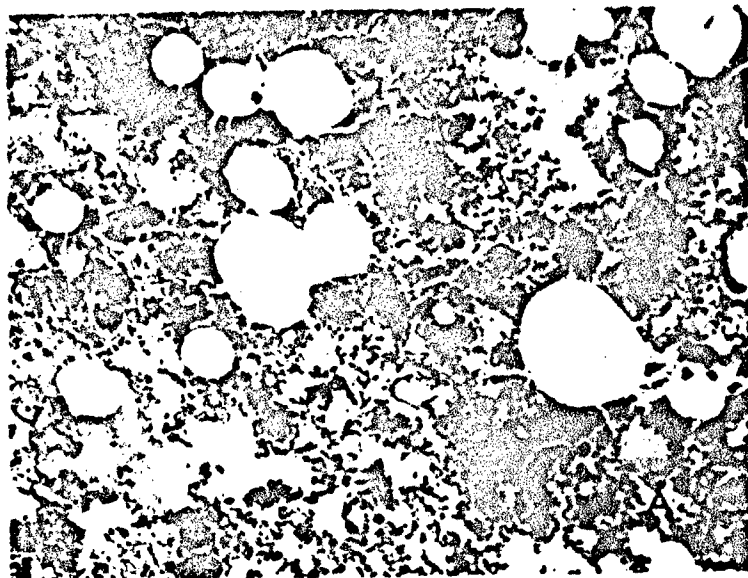


FIGURE 8. LUNG (ACC. 882, 500-SPORE GROUP) HEMORRHAGIC PNEUMONITIS WITH EDEMA, PROTEIN-LIKE PRECIPITATE, & NUMEROUS BACILLI. H & E (A) X400 (B) X300.

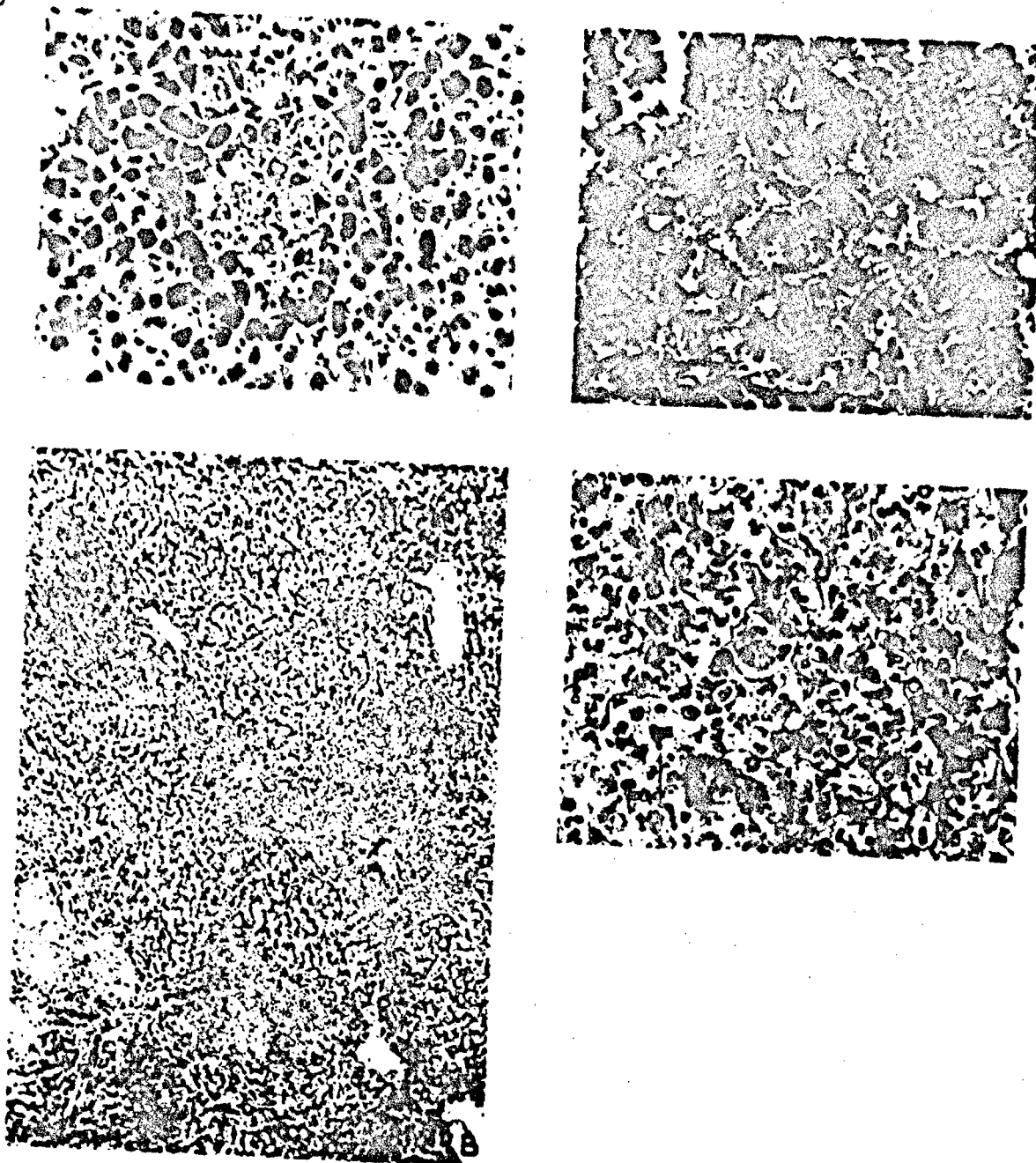


FIGURE 7. LIVER, HEPATIC NECROSIS, H & E
 (A) SMALL, ISOLATED, WITH NO
 SIGNIFICANT INFLAMMATORY REACTION
 (ACC. 843, 5000-SPORE GROUP, DEAD
 DAY 5) (B) X310. EXTENSIVE,
 RESEMBLING ACUTE YELLOW ATROPHY
 (ACC. 831, 500-SPORE GROUP, DEAD
 DAY 8) (C) X71 (D) FOCAL, WITH
 NUMEROUS BACILLI & MODERATE TO
 MARKED INFLAMMATORY REACTION. X240
 (500-SPORE GROUP C: ACC. 831,
 D: ACC. 834)

The alveolar walls were on occasion thickened by an irregular lining of homogeneous, fibrinous, acellular, pink material, which at times took on a hyaline appearance. Some of these alveolar spaces were filled with homogeneous or faintly granular proteinaceous eosinophilic material containing a variable number of erythrocytes (Figure 6).

Damage to bronchial epithelium was not seen outside of the lung mite lesions. Fibrinous pleuritis associated with a mild cellular response and hydrothorax was seen on occasion. Bacterial thrombi were observed in several instances.

Liver: The injury to the liver varied from congestion and edema to degeneration and necrosis, invariably associated with evidence of septicemia. The severity and occurrence of the hepatic lesions and their relationship to the time post-challenge and dose are shown in Tables III and IV.

Liver necrosis and hepatocellular destruction did not follow any specific pattern. There were small areas, not always sharply circumscribed, in which the hepatic cells were in various stages of degeneration and necrosis. In these areas the liver cells were either atrophic or swollen, even though they had distinct margins and a pale granular or partly hyalinized cytoplasm (Figure 7). The nuclei were frequently pyknotic with varying degrees of karyolysis and karyorrhexis. Necrosis was seldom diffuse. Essentially there was focal degeneration and destruction of hepatic cells frequently associated with mild inflammatory reaction.

In one instance only (No. 812) was there massive diffuse necrosis in which hepatic parenchyma was destroyed over extensive areas. This massive destruction of hepatic cells, a condition resembling "acute yellow atrophy," was accompanied by infiltration of inflammatory cells although the architectural pattern was generally well preserved. This condition was most marked about the peripheral parts of the lobules and was chiefly accompanied by mononuclear cells, lymphocytes, plasma cells with a few neutrophils and occasional eosinophils.

In general, regardless of the degree of destruction of the hepatic cells, the preserved sinusoids were congested and contained varying numbers of bacilli and inflammatory cells. Bacillary capsules were strongly PAS positive. In addition, the amount of PAS positive material in Kupffer cells was increased and some PAS positive material was observed within the hepatic cells (see discussion). The bile ducts were generally unaffected. The areas of necrosis, especially when small, could be central, midzonal or peripheral. Often a small necrotic area especially in its early evolution, was not accompanied by inflammatory reaction. Most of these necrotic lesions were accompanied by fatty metamorphosis and numerous bacilli; hemorrhages were rarely seen.

Gastrointestinal Tract: Most of the monkeys in this series had lesions of oesophagostomiasis and strongyloidosis. Bacilli were frequently found within these lesions. In one instance, an acute ulcer of the small intestine possibly associated with strongyloidosis, contained a large number of bacilli (Figure 8). In another case, numerous bacilli were found in an area of focal peritonitis associated with oesophagostomiasis. A third case of perforated chronic ulcer of the colon, containing bacilli, was also associated with oesophagostomiasis. In two of these cases ascites was observed.



FIGURE 8. INTESTINE (ACC. 814, 40,000-SPORE) ACUTE ULCER, POSSIBLY PARASITIC, WITH MASSIVE ACCUMULATIONS OF BACILLI. H & E. (A) X14, (B) X365.

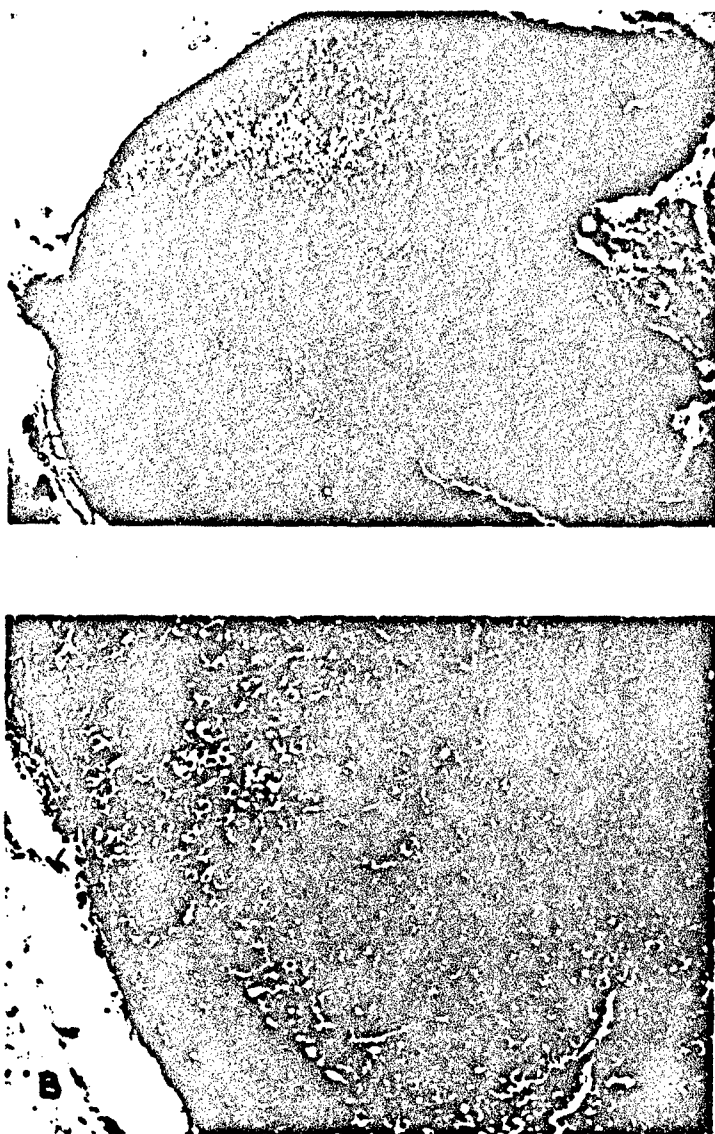


FIGURE 9. ADRENAL GLAND (ACC. 870, 5000-SPORE GROUP)
CORTICAL HEMORRHAGE, WITH NECROSIS, MULTIPLE.
H&E (A) X17. (B) X88.

TABLE III. SUMMARY OF MICROSCOPIC PATHOLOGY OF EXPERIMENTAL INTRADERMAL^{a/} ANTHRAX IN MONKEYS

DOSE (Spores)	MONKEY NUMBER (Acc. No.)	DAY OF DEATH	LYMPHATIC SYSTEM			RESPIRATORY SYSTEM				GI Tract	Adrenal	Bone Marrow	Cellulitis
			Artery	Others	Spleen	Hemo- rhages	Edema	Pneumo- nitis	Liver				
500	809 (642)	17	+++ ^{a/}	+	++	+	++	+	+++	-	-	-	+++
	810 ^{b/} (634)	10	++	-	++	-	-	-	+++	+	+	-	+++
	812 (631)	8	+	-	+	-	-	-	+++	-	±	-	+++
	C-9 ^{c/} (662)	3	++	+	++	+++	+	+++	++	+	-	+	+
5,000	722 ^{c,d/} (644)	5	++++	+++ ^{d/}	++	-	-	+	+	-	-	+	++
	734 (671)	5	+	+	-	-	-	-	++	-	-	-	++
	705 (643)	5	+	-	++	+++	+++	+	+++	-	+	+	+
	766 ^{b/} (670)	3	++	++	++	-	-	-	++	-	++	-	++++
10,000	892 (899)	5	+++	+++ ^{d/}	++	-	-	+	++	-	++	+	++++
40,000	678 (814)	4	+++	++	++	-	-	-	+	++	++	-	++++

a. Intradermal except Monkey No. 892 - Subcutaneous.

b. Kidney changes: -(-)

c. Cardiovascular changes: -(-)

d. CNS changes: -(-)

TABLE IV. HEPATIC CHANGES IN EXPERIMENTAL INTRADERMAL^{a/} ANTHRAX IN MONKEYS

DOSE (Spores)	MONK NO. (Acc. No.)	CON- GEST- ION	EDEMA (Serous Hepa- titis)	DIS- TURBANCE OF ARCHI- TECTURE	DEGEN- ERATION AND ATROPHY	INDI- VIDUAL CELL NECRO- SIS	FOCAL NECRO- SIS	PAS-POSITIVE MATERIAL		SUMMARY GRADING
								Liver Cells	Kupffer Cells	
500	809 (642)	+++	+++	+++	+++	++	+++	++	+	+++
	810 (634)	+++	+++	+++	+++	+++	++	+	++	+++
	812 (631)	++	++	+	++	+	++++ ^{b/}	+	++	++++
	C-9 (662)	+++	+++	++	++	-	-	++	+	++
5,000	723 (644)	+	++	±	-	±	+	±	+	+
	743 (671)	+++	++	+	++	+	++	+	+	++
	705 (643)	+++	+++	+++	+++	+++	+++	+	++	+++
	766 (670)	++	++	++	++	++	++	-	++	++
10,000	892 (899)	++++	++++	+++	++	++	++	+	++	++
40,000	678 (814)	+	+	+	+	+	+	+	++	+

a. Intradermal except Monkey No. 892 - subcutaneous.

b. Extensive necrotizing hepatitis with little modification of the remaining hepatic parenchyma.

Endocrine Organs: The adrenal was the only gland frequently involved. The adrenal lesion was essentially cortical, characterized by isolated or multiple hemorrhages with or without necrosis and usually with numerous bacilli (Figure 9). Bacterial thrombi, similar to those encountered in the liver, lungs and occasionally elsewhere, were also observed.

Pancreas, pituitary, testis, thyroid, parathyroid glands were generally spared. No lesions were detected in these organs.

Central Nervous System: Involvement of the central nervous system was seen in only one of this series of animals. In this instance (No. 723) an extensive hemorrhagic meningitis with an intense neutrophilic infiltration into the meninges, a concomitant necrotizing vasculitis and necrotizing lymphadenitis (tracheobronchial and hilar), were recorded (Figure 10A & B).

Genitourinary System: Lesions of the genitourinary tract were minimal. In two instances there were focal hemorrhages with bacilli in the ovaries. The kidneys were spared except in two cases, isolated, small, and rather minute foci of cortical necrosis were observed. Other kidneys had varying numbers of bacilli within the glomerular tufts with no significant pathologic changes (Figure 11).

Cardiovascular System; Heart: Although the myocardium and the large blood vessels were not affected, definite changes in the walls of some blood vessels were observed. In two instances, focal small myocardial hemorrhages were seen. In one instance, a mild myocarditis was observed. Minute interstitial foci of necrosis, composed of degenerating myocardial fibers, were seen within this lesion. In this case, the capillaries were dilated, and there were focal extravasations. A few small clumps of perivascular inflammatory cells, chiefly mononuclear, were also present. The walls of some capillaries revealed focal degeneration or necrosis, with numerous bacilli within and outside of the lumen.

Blood Vessels: The principal lesions of blood vessels were the bacterial thrombi seen in many organs associated with septicemia and a necrotizing lesion of the vessel walls observed in several organs. This lesion generally observed in association with necrosis, hemorrhage, and numerous bacilli, was characterized by a frank necrosis or fibrinoid-like degeneration of the vessel walls (Figures 3 and 10).

Thymus: Anthrax bacilli had no injurious effects upon the thymus. It was consistently spared.

Bone Marrow: The bone marrow was generally cellular. In some cases there were congestion and increased numbers of megakaryocytes. In other animals the megakaryocytes underwent degeneration or partial necrosis with pyknosis and fragmentation of nuclei (Figure 12). Occasionally small foci of necrosis infiltrated with inflammatory cells, chiefly mononuclear, were found. Varying numbers of bacilli were present.

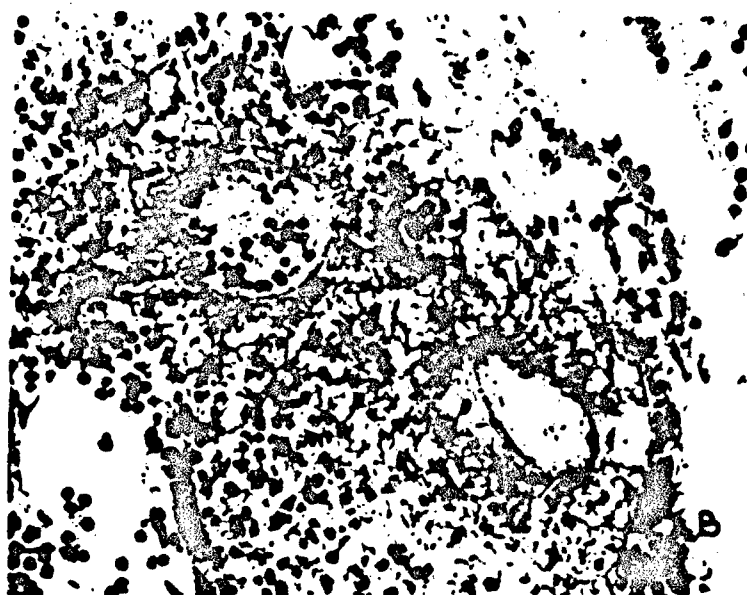


FIGURE 10. MENINGES. (ACC. 844, 5000-SPORE GROUP, DEAD DAY 5)
MASSIVE, HEMORRHAGIC MENINGITIS, WITH NUMEROUS
BACILLI & WIDESPREAD NECROTIZING VASCULITIS. H & E
(A) X100, (B) X300.

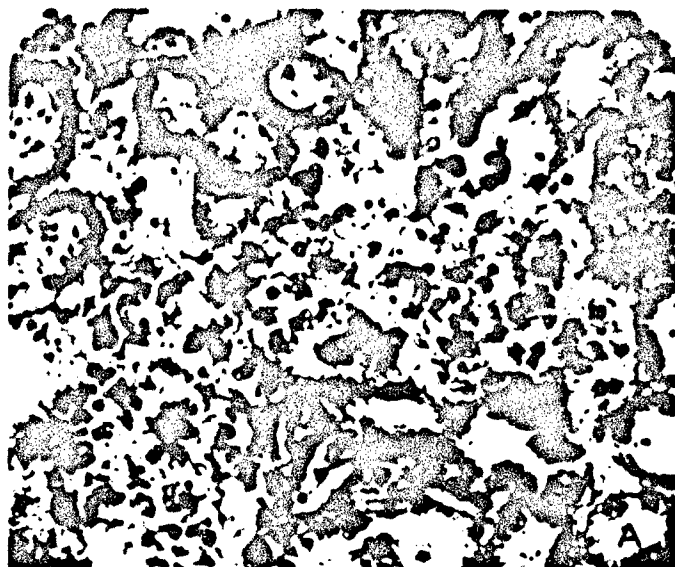


FIGURE II. KIDNEY (ACC. 834, 5000-SPORE GROUP, DEAD DAY 5) (A) FOCAL CORTICAL NECROSIS WITH NUMEROUS BACILLI. H&E X250. (B) GLOMERULUS, OF SAME KIDNEY, NUMEROUS BACILLI WITHIN GLOMERULAR TUFT, WITHOUT BEING SIGNIFICANTLY AFFECTED. H&E X374.



FIGURE 12. BONE MARROW (ACC. 844, 5000-SPORE GROUP)
TWO FOCI OF NECROSIS & DEGENERATING
MEGAKARYOCYTES. H&E X88.

TABLE V. SUMMARY OF PRINCIPAL LESIONS^{a/} CONTRIBUTING TO DEATH
IN EXPERIMENTAL INTRADERMAL^{b/} ANTHRAX MONKEYS

DOSE (Spores)	MONKEY NO. (Acc. No.)	DAY OF DEATH	LESIONS	
			EARLY (1-5 days)	LATE (3-17 days)
500	809 (642)	17	-	Extensive cellulitis and myositis. Necrotizing hepatitis.
	810 (634)	10	-	Cellulitis. Necrotizing hepatitis, moderate.
	812 (631)	8	-	Massive necrotizing hepatitis. Cellulitis.
	C-9 (662)	3	Hemorrhagic pneumonitis.	
5,000	723 (644)	5	Necrotizing hemorrhagic meningitis ^{c/} . Hydro- thorax.	-
	743 (671)	5	Hydrothorax. Necrotizing hepatitis, moderate.	-
	705 (643)	5	Hemorrhagic pneumonitis.	-
	766 (670)	3	Extensive cellulitis. Adrenal hemorrhage and necrosis.	-
10,000	892 (899)	5	Pneumonitis. Extensive cellulitis. Adrenal hemorrhages.	-
40,000	678 (814)	4	Extensive cellulitis. Adrenal hemorrhages.	-

a. Septicemia, lymphadenitis and splenitis were present in all cases.

b. Intradermal except Monkey No. 892 - subcutaneous.

c. Hemorrhagic meningitis was also seen in two other animals, from another series, that died on days 4 and 6. See "CNS" under discussion.

IV. DISCUSSION

Based on this systematic study of the organs of the monkeys dying from septicemia and its complications, it appears that there was no uniform histopathologic picture. Septicemia, lymphadenitis and splenitis were present in all cases.

As has been pointed out, all monkeys died following the challenge. Although the number of animals was small in each group, an attempt was made to correlate the time of death with the size of challenge administered. The range of survival time for the 500-spore animals was 3 to 17 days with a mean of 9.5 days. The range of survival time for 5,000-spore animals was 3 to 5 days with a mean of 4 days. All animals died with a septicemia.

The principal lesions in this series of animals are shown in Table V and summarized as follows: frequently (4/7), in the group with early deaths (1 to 5 days), the animals died of respiratory involvement, either hemorrhagic pneumonia or massive hemorrhage; the animals dying later (3 to 17 days) developed extensive cellulitis at the site of inoculation often accompanied by necrotizing hepatitis.

The variability of the pathologic responses of this host to this infection is indeed remarkable. Each animal and every tissue appeared to respond differently. In the same animal some organs were rich in bacilli, others spared. Although the capillaries and blood vessels exhibited varying numbers of bacilli, the lung parenchyma for example was sometimes spared.

The lymphatic system and blood appeared to be the first targets in which the bacilli were trapped even though they showed the greatest variation in histologic pictures. Both appeared to play a role in the dissemination of the organisms from the site of inoculation, and there was no evidence that one is the preferred initial route over the other. Some lymph nodes in a single animal were spared whereas others contained myriads of bacilli. This discrepancy was even more prominent from one animal to another. The spleen differed from the lymph nodes in that it was invariably involved, and contained myriads of bacilli.

A classification of the systems and organs of M. mulatta according to decreasing sensitivity of B. anthracis infection initiated by intradermal inoculation may be presented as follows:

1. Most sensitive: blood, spleen, and lymphatic system.
2. Moderately sensitive: liver, adrenals, and occasionally bone marrow.
3. Mildly sensitive (occasionally involved, especially when there was a pre-existing lesion, such as a parasitic lesion or ulcer): gastrointestinal tract, respiratory tract, genitourinary system, cardiovascular system, and central nervous system.

4. Extremely resistant (no bacilli detectable in multiple sections made of these organs): thymus, endocrine glands (except adrenals), salivary glands, skin, and skeletal muscle (excluding the site of inoculation).

While inflammatory cells, neutrophils, lymphocytes, and monocytes, were present in the spleen, lymph nodes, lungs, and less often in liver, bone marrow, and adrenals, their numbers were not prominent. In general the extent of the inflammatory reaction was minimal or mild. The skin and subcutaneous tissues were the only tissues in which the inflammatory reaction was prominent in those cases that developed a cellulitis at the site of inoculation.

The dominant histologic features of parenteral anthrax were necrosis and hemorrhage with or without inflammatory reaction. The spleen and lymph nodes were the two organs in which this destruction was most conspicuous. Hemorrhage and necrosis generally destroyed a part or all of a lymph node; in some instances the architectural pattern was almost totally destroyed. The same picture was seen in the spleen.

Massive pulmonary infection, lobar pneumonia or extensive consolidation were uncommon. However, focal, petechial, or diffuse hemorrhages and hemorrhagic pneumonitis occurred, particularly in animals dying early.

Focal necrosis, isolated or multiple, often associated with hemorrhage, was observed in the adrenal cortex on several occasions. The integrity of the adjoining tissue, however, was always maintained. Diffuse destruction of adrenals was not observed. Liver necrosis and hepatocellular destruction of varying intensity and distribution were seen. The amount of PAS positive material was significantly increased in Kupffer cells, and some strongly PAS positive droplets were also shown in hepatic cells. According to Popper et al., the presence of PAS positive material in hepatic cells and its increase in Kupffer cells indicates a disturbance of liver function and impairment of metabolic activity. These authors state further that the demonstration of PAS positive material in the liver is a valuable method for recognizing the intensity of liver cell damage.

Although the central nervous system is involved under certain circumstances¹, it is not generally affected in intradermally infected monkeys. In one instance only was there extensive necrotizing hemorrhagic meningitis in a monkey challenged with 5,000 spores. This animal had an in-dwelling cardiac catheter.

In another series of animals receiving subcutaneous challenges and not included in this study, cases of hemorrhagic meningitis were seen.

V. SUMMARY

This study was undertaken to ascertain the detailed pathologic changes of parenterally induced anthrax in unmodified M. malletta and to discuss its pathogenesis. A summary of the principal lesions essentially due to septicemia and its consequences was presented. The dominant histologic features of intradermally induced anthrax in this host were necrosis and hemorrhage with or without inflammatory reaction.

A sequence of events may be reconstructed as follows (Figure 13): Germination of spores probably occurs at the site of inoculation. Bacilli multiply and produce a local lesion at this point. The organisms invade the lymphatics and blood vascular system; upon arriving at the spleen and lymph nodes, they cause extensive hemorrhage and necrosis in these tissues. With a high infecting dose of spores, an overwhelming septicemia develops early (1 to 5 days), frequently with respiratory complications due to lesions of the pulmonary parenchyma or fluids in the pleural cavity. With small doses, death generally occurs later (3 to 17 days, mean 9 days) with frequent lesions in the viscera, i.e., liver and adrenals.

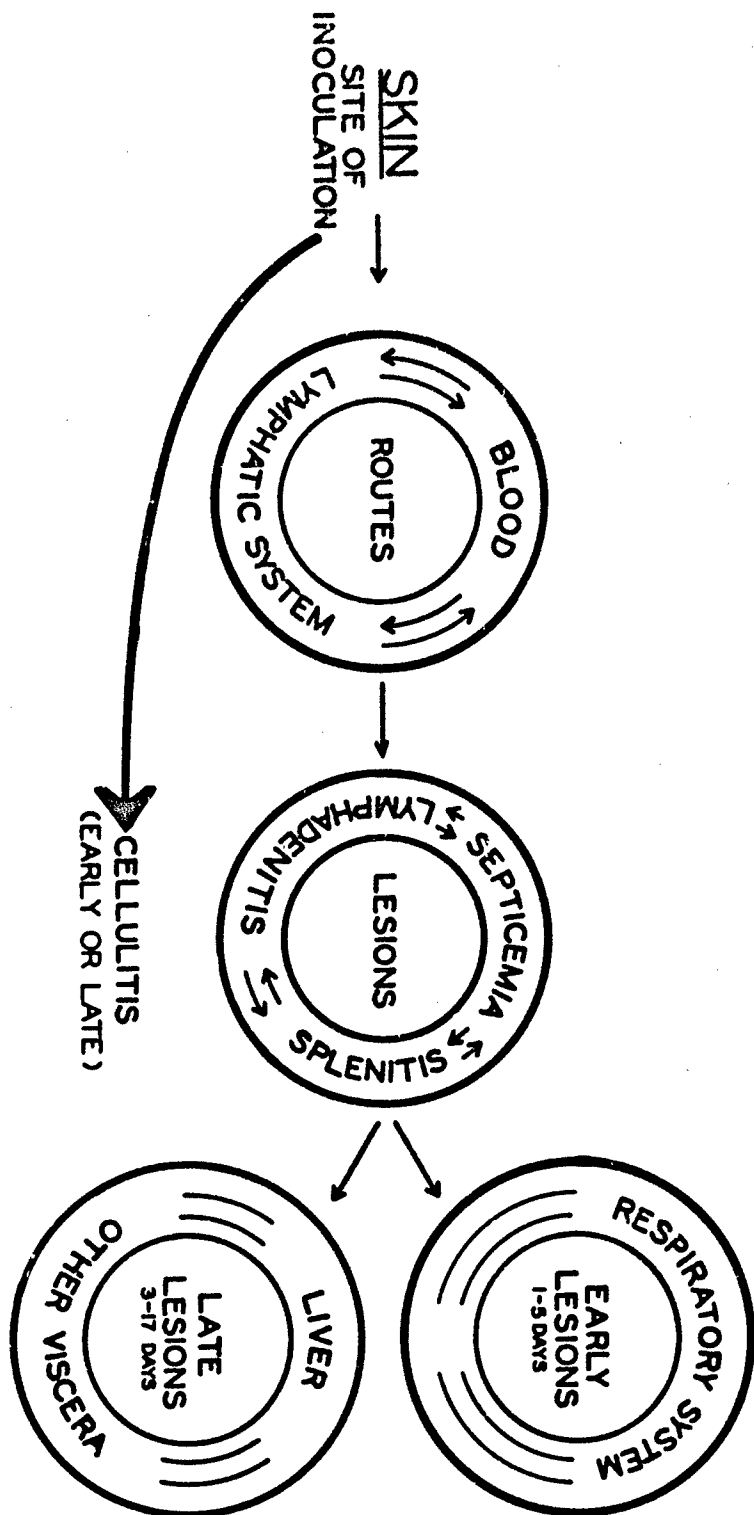


FIGURE 13. DIAGRAMMATIC PRESENTATION OF PATHOGENESIS OF EXPERIMENTAL INTRADERMAL ANTHRAX IN MONKEYS.

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STUDIES ON BACILLUS ANTHRACIS

PART 4

PATHOLOGY OF EXPERIMENTAL RESPIRATORY ANTHRAX IN MACACA MULATTA
(Gleiser, Berdjia, Hartman and Gochanour)I. INTRODUCTION

The pathology of terminal intradermal anthrax in the unmodified Macaca mulatta is described elsewhere^{1/}. The principal lesions observed were hemorrhage and necrosis involving a wide variety of organs and systems, cutaneous, lymphatic, vascular, hepatic, endocrine, and central nervous.

Experimental respiratory anthrax has been studied in a wide variety of animal hosts in an attempt to elucidate the pathogenesis of respiratory anthrax in man. Young^{2/} reported studies in guinea pigs, mice, dogs, rats, and monkeys but omitted pathology for the monkey. Albrink^{3/} described pathology of respiratory anthrax in three chimpanzees and Barnes^{4/} and Rosa^{5/} reported on the pathogenesis in guinea pigs, rabbits, and mice.

It is the purpose of the present studies to determine the pathologic picture of terminal respiratory anthrax in the unmodified M. mulatta, and further to determine if the classical inhalation anthrax, "Woolsorters' Disease" of man so vividly described by Greenfield^{6/} in 1831 and "Inhalationsmilzbrand" by Fraenkel^{12/} in 1925, could be reproduced in this host. This report deals with our pathologic findings and compares them with those of intradermal anthrax in this host.

Any description of the pathologic changes in an experimental respiratory infection must be preceded by a very brief commentary on the normal anatomy of the lung.

The area of primary concern is that portion of the respiratory tree that begins with the terminal bronchiole, has no cartilage, and has an abundance of muscle and columnar ciliated epithelium (Figure 1). This portion divides into the respiratory bronchioles which give rise to individual alveoli called respiratory alveoli. The transition to cuboidal non-ciliated epithelium occurs at this level. The respiratory bronchiole divides into the alveolar ducts which are lined by low cuboidal or squamous type epithelium. The atria, somewhat spherical cavities arising from the alveolar duct (3 to 6 per duct), are lined with flat respiratory epithelium and give rise to the alveolar saccule and the pulmonary alveoli.

The respiratory alveoli which arise directly from the respiratory bronchiole are of particular importance in any consideration of the pathogenesis of a respiratory infection because they are the only alveoli which are accompanied by lymphatics. Lymphatics according to Miller^{7/} are not found beyond the alveolar ducts. Therefore there are no lymphatics at the level of the alveolar saccules and the terminal pulmonary alveoli.

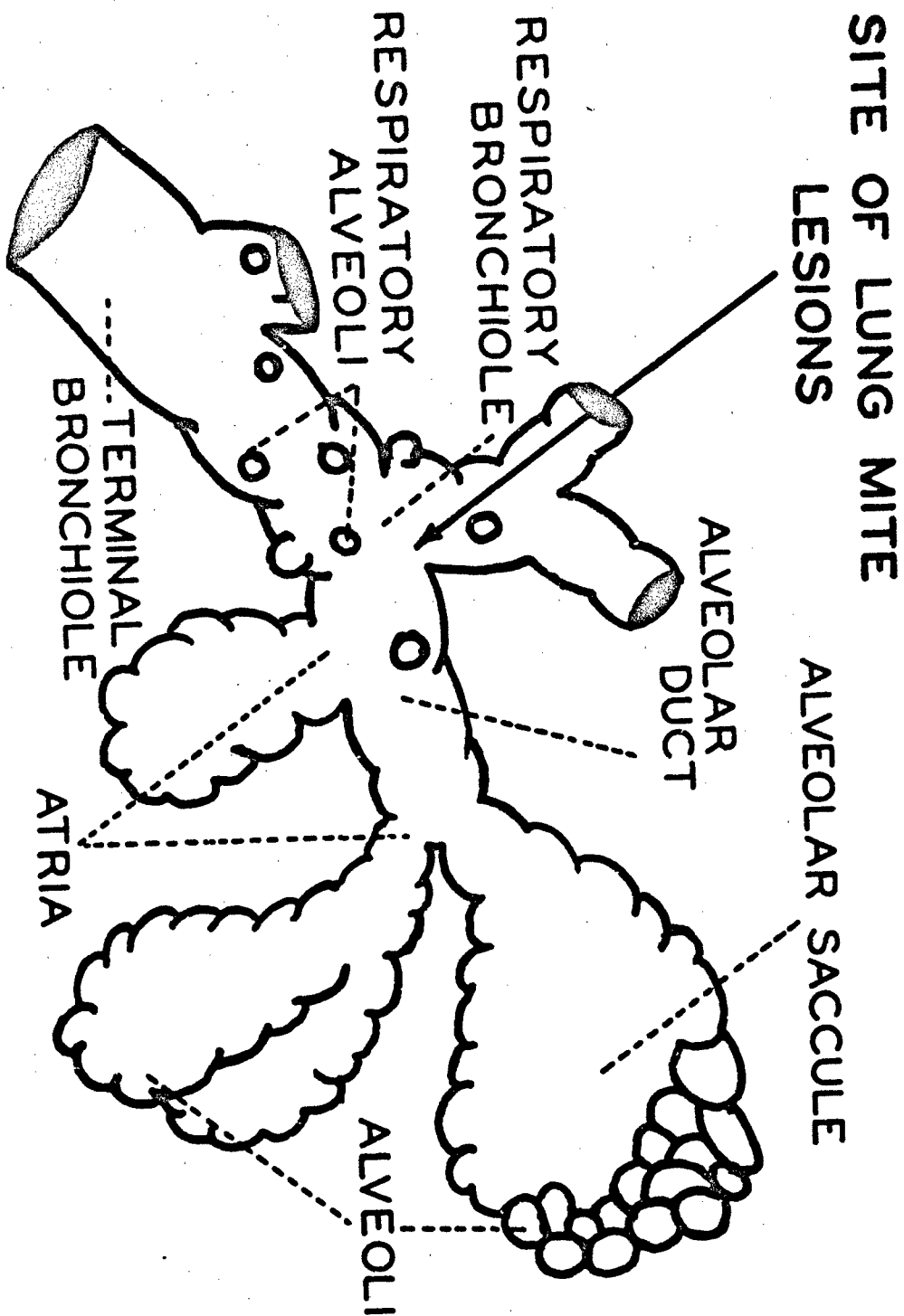
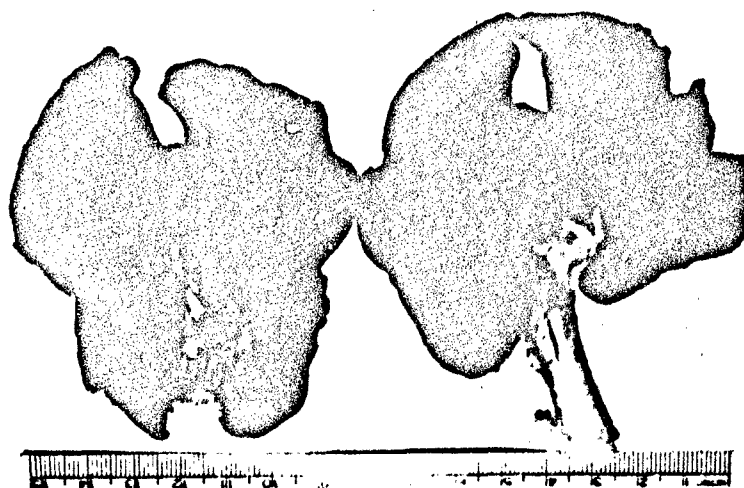


FIGURE 1. DIAGRAM SHOWING PRINCIPAL SITE OF LUNG MITE LESIONS



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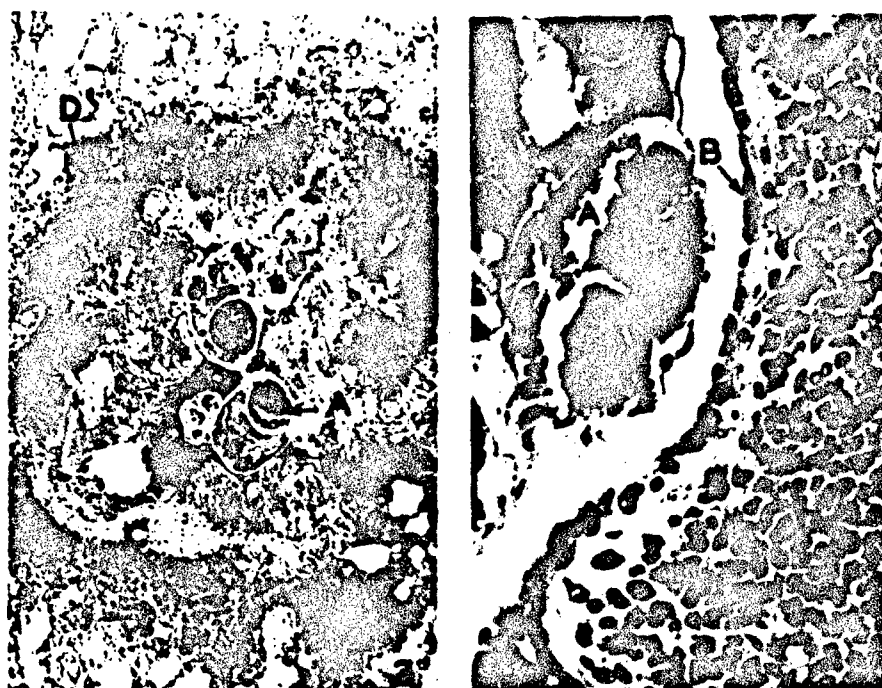


FIGURE 2. ACC. 1097 - LUNG MITE LESIONS AS USUALLY SEEN GROSSLY IN M. MULATTA.

FIGURE 3. ACC. 25 - LUNG MITE LESION IN M. MULATTA: (A) MITE; (B) PERIBRONCHIOLAR LYMPHOID HYPERTROPHY; (C) MUSCULAR HYPERTROPHY; (D) PARASITIC PIGMENT X25

FIGURE 4. ACC. 25 - LUNG MITE LESION. (A) PARASITE; (B) FLATTENED BRONCHIOLAR EPITHELIUM; (C) PERIBRONCHIOLAR LYMPHOID ELEMENTS, MACROPHAGES, & OCCASIONAL EOSINOPHIL. X360.

The question of the presence of an epithelial lining in the alveoli is beyond the scope of this study. The interested reader is referred to texts on the anatomy of the primate lung^{7,8}.

Mention also should be made at this time of the lung-mite an ever-present parasite in the pulmonary tree of the *M. mulatta* and the lesion it produces. The lung mite, *Pneumonyssus simicola* and related species, has a reported incidence of 80 to 100 per cent in this host^{9,10}. The lesions, described by Innes *et al*¹⁰ are readily found at autopsy as subpleural, discrete, generally depressed, yellow lesions varying in size from 1 to 2 mm (Figure 2). They are found in all portions of the lobes. Sometimes they have a halo of congestion. The microscopic picture of the lung mite lesion is variable, probably being a function of the age of the lesion. The lesion occurs in bronchioles at a level where cartilage is absent. It is essentially a bronchiolitis accompanied by muscular hypertrophy, lymphoid hyperplasia, variable degrees of eosinophilia, much pigment of parasitic origin, many pigment-laden macrophages, occasional giant cells, thickening of the bronchiolar wall, destruction of the lining epithelium, and some involvement of the adjacent pulmonary parenchyma (Figures 3 and 4). The mite itself is frequently seen in the lumen or in the wall of the bronchiole. The lesion is discrete, but not encapsulated. These lesions, involving the portion of the respiratory tree intimately concerned in experimental respiratory infections, must be borne in mind in any consideration of such infections and their pathogenesis.

The true lymph nodes in the pulmonary cavity of *M. mulatta* include the hilar, mediastinal, and tracheobronchial. The hilar nodes are discrete and are situated in the hilus of each lung. The mediastinal nodes are multiple, small, discrete nodes situated along the trachea. The tracheobronchial nodes form a cluster of small nodes at the bifurcation of the trachea. These true lymph nodes for sake of brevity will be referred to as "intrathoracic nodes."

II. MATERIALS AND METHODS

Animals used for these studies were young, 2 to 4 kg, immature *M. mulatta* of both sexes, in good states of health and nutrition; they had been tuberculin tested and screened radiographically for chest pathology. Base-line temperatures were generally taken for two days prior to exposure.

A spore suspension of the Vollum-189 strain of *Bacillus anthracis* prepared in July 1957 was used in these studies. The spore concentration at the time of preparation was approximately 5×10^{10} cells/ml. The material had been stored at 5°C in 5 per cent phenol. Working suspensions were heat-shocked approximately 48 hours prior to aerosolization by incubation at 60°C for 30 minutes. Plate counts made on the original suspension from time to time have shown no significant loss in viability. Likewise, parenteral guinea pig titrations have failed to show any loss of virulence of the spore suspension during the period of storage.

Animals were exposed to a dynamic cloud generated in a modified Henderson apparatus¹¹. A primary aerosol was generated at the rate of 8 L/min by employing a spray head producing particles, 80 per cent of which were 2 μ or

less in diameter. The primary aerosol was diluted with tempered air at a relative humidity of 80 per cent at the rate of 20 standard ft³/min. The aerosol was drawn past the animals' faces in a closed exposure port and evacuated from it through glass impingers containing distilled water. Estimation of aerosol concentration presented to the animals was made by replicate standard plate counts of appropriate dilutions of impinger fluids. Check virulence titrations of suspensions used for aerosolization and impinger fluid were made in guinea pigs. Dosages presented to the animals were varied by appropriately diluting the spore suspension to be aerosolized or by varying the time of exposure. In this series, presented doses varied from 1×10^4 to 3×10^6 spores. Table I presents the dose distribution data.

TABLE I. DISTRIBUTION OF ANIMALS EXPOSED TO RESPIRATORY DOSES OF SPORES
B. ANTHRACIS (VOLLUM-189) IN UNMODIFIED M. MULATTA

DOSE RANGE	NUMBER OF <u>M. MULATTA</u>
1×10^4 to $< 5 \times 10^4$	4
5×10^4 to $< 5 \times 10^5$	6
5×10^5 to $< 1 \times 10^6$	16
1×10^6 to $< 3 \times 10^6$	2

Post-exposure observations consisted of at least twice daily examination of the animals; including pulse, respiration, and temperature and visual observation of behavior. Blood cultures were made at varying intervals, in some instances daily, in others, every other day.

Autopsies in most instances were performed within two hours of death. In a few instances, moribund animals were sacrificed by exsanguination. Blood smears were prepared at time of autopsy, stained with crystal violet, and examined for the presence of bacilli. In these rare instances when gross bacteremia was not evident upon examination of a stained blood smear, blood cultures were made by spreading approximately 0.2 ml of blood on the surface of duplicate nutrient agar plates; these were incubated at 35°C for 24 hours and examined for typical colonial growth.

Tissues were collected in 10 per cent saline-formalin neutralized by precipitated CaCO₃. Tissue sections cut at 6 μ were routinely stained with hematoxylin and eosin. H&E was usually satisfactory for demonstration of bacilli in sections, although when necessary the Brown and Brenn stain was employed.

III. RESULTS

A. CLINICAL RESPONSES

The clinical responses of the unmodified M. mulatta to respiratory exposures of heat-shocked spores of B. anthracis may be briefly described as undramatic and inconsistent. Some animals developed fevers, others did not; some animals developed respiratory distress prior to exitus, some, marked depression, and a few, convulsions. Emesis prior to exitus was observed on occasion. Diffuse subcutaneous edema of portions of the head or chest was observed in two instances. All infected animals died with terminal bacteremia, which in most instances was massive, ascertainable merely by microscopic examination of a stained blood smear.

All animals known to have been successfully exposed by aerosol to more than 1×10^4 spores were infected with three exceptions: one animal on autopsy proved to have generalized tuberculosis with no evidence of anthrax infection. The other two animals were exposed to low doses, 10,100 and 10,400 spores.

Time of death appeared to be dose-dependent. This relationship became apparent when the 28 animals in this study were divided arbitrarily into two groups relative to their presented doses: $< 500,000$ and $\geq 500,000$ spores. The survival time range for the lower dose group was 3 to 20 days with a mean of 7.5 days; for the higher, 2 to 5 days with a mean of 3 days.

B. GROSS PATHOLOGY

The principal lesions observed at autopsy are shown in Table II. It is apparent that the incidence of some lesions, mediastinitis, meningitis, and lung lesions is dose-related. The high incidence of intrathoracic lymphadenopathy in both dose groups is particularly striking; these are the lesions that characterize respiratory anthrax infection in M. mulatta and are worthy of special consideration.

Lymphatic System: The gross changes in the lymphatic system in respiratory anthrax are essentially similar to those of intradermal anthrax infection¹. Splenomegaly was frequently seen. Edema, hemorrhage, and necrosis of various lymph nodes were often observed, most frequently and most intensely in the intrathoracic nodes. At times these nodes were significantly enlarged and massively hemorrhagic; at other times they were only hemorrhagic. Sometimes these nodes only were the site of gross lesions. This was particularly true in the animals dying quickly after exposure to a large number of spores.

Pleural Cavities: Hydrothorax was an inconstant finding; when found severity was variable.

TABLE II. INCIDENCE OF PRINCIPAL GROSS LESIONS IN TERMINAL RESPIRATORY ANTHRAX IN THE UNMODIFIED M. MULATTA

GROSS LESIONS	DOSE OF <u>B. ANTHRACIS</u> SPORES (VOLLUM-189)			
	< 500,000		≥ 500,000	
	10 Monkeys No. (Z)		18 Monkeys No. (Z)	
Mediastinitis	2 (20)		10 (56)	
Meningitis, hemorrhagic	2 (20)		6 (33)	
Hydrothorax	3 (30)		4 (22)	
Hemorrhages, lung	2 (20)		12 (67)	
Hemorrhagic parasitic nodules, lung	2 (20)		12 (67)	
Hemorrhages, adrenal	3 (30)		8 (44)	
Hemorrhages, other (retroperitoneal, G. I., etc.)	3 (30)		7 (39)	
Lymphadenopathy, intrathoracic	7 (70)		13 (72)	
Splenomegaly	2 (20)		8 (44)	
Ascites	1 (10)		3 (17)	

Mediastinum: Edema of the mediastinal tissues with some hemorrhage (Figure 21) was a frequent finding. This lesion was characterized by a varying degree of widening of the mediastinum and its structures associated with the hemorrhagic lymphadenopathy already described. The tissues also had an abnormal glistening sheen. Massive hemorrhagic mediastinitis was not observed in unmodified respiratory anthrax, with one exception (Monkey No. 927): this animal, exposed to low aerosol dose, died on day 11 with gross hemorrhagic mediastinitis and meningitis. Such hemorrhagic mediastinitis was observed frequently in monkeys exposed via the respiratory route and then placed on antibiotic therapy which modified the course of the disease but did not spare the animal.

Respiratory System: The sinuses and nares were not examined in these animals. The respiratory tree beginning at the larynx was carefully examined. In no instance was a "primary anthrax lesion", either hemorrhagic as described by Greenfield⁶/, or ulcerative as described by Fraenkel¹²/, observed in the trachea or bronchi. Scattered patchy parenchymal hemorrhages and edema of varying intensity, as seen in intradermal anthrax, and, expected in any fatal bacteremia, were observed in a large number of animals.

"Lung mite" lesions were present in the lungs of practically all animals. Of particular interest and importance in this infection in this host were subpleural circumscribed nodular lesions seen in many of the pulmonary lobes (Figures 3 and 9). These lesions were 1 to 1.5 cm in diameter. They were firm to hard in consistency, and well circumscribed but not encapsulated. The subpleural surface of the lesion was usually dark or hemorrhagic throughout but in some instances the center was light grey and the periphery dark. The cut surface of the lesion was dark with a light center (Figure 6). The sectioned lesion was again found to be uniformly firm and well circumscribed. These lesions are listed as "hemorrhagic parasitic nodules" in Table II; their incidence also appears to be dose-dependant.

Central Nervous System: Hemorrhagic meningitis was observed in 33 per cent of the animals in the large dose group, and in 20 per cent in the low dose group (Table II). In some instances the lesion was uniformly diffuse and intense over the entire brain; in a few cases it was patchy and of varying intensity over the cerebral and cerebellar surfaces. The hemorrhagic process was confined to the meninges and did not extend into the brain proper. Edema of the brain, however, as evidenced by excessive softness and wetness, was a frequent concomitant finding.

Other Gross Findings: Hemorrhages were found in other locations with inconstant frequency; retroperitoneal and adrenal hemorrhages were most frequent. Other inconstant or rare findings were hydropericardium, edema of the pericardial sac, ascites, edema of the subcutaneous tissues of portions of the head and chest, and gelatinous retroperitoneal edema. In one menstruating female hemorrhagic cellulitis of the nipples and mammary tissues was observed. Cellulitis of traumatic origin in the inguinal regions and the inner aspect of the upper thighs resulting from repeated venipuncture of the femoral sinuses was seen in some of the animals.

Lesions of oesophagostomiasis (a hookworm parasitism by the nematode, *Oesophagostomum*) were observed in a large percentage of the animals in the mesentery and in the wall of the large intestine, particularly at the point of attachment of the mesentery to the intestine.

C. MICROSCOPIC PATHOLOGY

Lymphatic System: The histologic changes seen in the lymphatic tissues were the same as those described for inoculation anthrax¹. The principal changes were: (1) necrosis of the lymphatic elements in the spleen and lymph nodes of varying degrees, from minimal to massive (Figure 15); (2) hemorrhage of varying intensity, at times, massive (Figure 16); (3) leukocytic, monocytic and neutrophilic, infiltration, also of varying intensity, perhaps related to the degree of necrosis; (4) variable numbers of bacilli in vessels and sinusoids, large numbers occurring in those cases where lymphatic necrosis was massive (Figure 17); (5) complete necrosis of the walls of small blood vessels, a lesion described by Berdjis *et al.*² as "necrotizing vasculitis," again occurring in those nodes in which necrosis was most marked (Figure 27); (6) deposition of small amounts of fibrin, especially in association with marked necrosis (Figure 16); and (7) phagocytosis of nuclear debris, resulting from

lymphatic necrosis, erythrophagocytosis, and occasional phagocytosis of bacilli by monocytes (Figure 18). Phagocytosis of spores was suspected in many instances, but never satisfactorily confirmed by special staining techniques^{3/}. These changes were observed most frequently and with greatest intensity in the intrathoracic nodes. Not infrequently these nodes were severely involved, whereas other nodes (axillary, inguinal, submaxillary, cervical, mesenteric) examined were involved to a minimal degree. In these other nodes one saw only minimal lymphatic destruction and few bacilli in sinusoids. The architectural integrity of these nodes was well preserved.

In six cases there was minimal lymph node involvement but massive septicemia and extensive splenic involvement. In these animals the tracheobronchial and hilar lymph nodes were amazingly intact. In each of these animals there were pulmonary lesions, either hemorrhages, pneumonia or necrotizing bronchiolitis due to superinfection of the lung mite lesions with B. anthracis.

Spleen: The changes in spleen were similar to those observed in lymph nodes. In addition, there was usually dilatation of the sinusoids which were frequently engorged. Erythrophagocytosis was seen frequently. The lymphatic necrosis was generally severe; at times, the spleen in its severely depopulated state would take on the appearance of a "bag of bacilli" with masses of bacillary forms enclosed by the splenic capsule. Bacilli in vessels were frequently observed (Figure 24).

Respiratory Tract: Edema of the larynx and trachea was seen in two instances. No tracheal or bronchial erosion or ulcer was seen. Pulmonary changes that may well be expected in a fatal bacteremia, i.e. foci of edema and hemorrhage, were seen. Patchy inflammatory infiltrates chiefly monocytic were also frequently observed.

Bacilli were often seen in the alveolar capillaries and larger blood vessels and sometimes free in the alveolar spaces, singly or in clusters. Pulmonary macrophages or histiocytes were also observed lying free in alveolar spaces.

The principal pulmonary lesion was described grossly as a discrete "hemorrhagic parasitic nodule." Histologically, it may be described as follows: the site was the bronchiole parasitized by the lung mite (Figure 7). The mite itself was seen on several occasions in the lumen of the dilated bronchiole (Figure 10). At other times, although the mite itself was not seen, the characteristic pigment and lymphoid and muscular hypertrophy were ample evidence of its presence (Figure 7).

The bronchiole frequently was tremendously dilated and the epithelial lining was flattened to such an extent that it looked like endothelium (Figures 7, 11 and 13). Sometimes the lumen of this dilated bronchiole was filled with homogeneous, eosinophilic, acellular, granular material that may have been serum or lymph (Figures 7, 10 and 11). Strands of fibrin traversed this material. At the periphery of the lumen there was frequently a pseudo-membraneous-like structure of a homogeneous, fibrinous, acellular, hyaline-like eosinophilic material (Figures 7, 10 and 11). In some places this fibrinous

material appeared to actually replace the bronchiolar epithelium, and in fact the wall (Figure 11). At these points the architectural integrity of the bronchiolar wall seemed to be completely obliterated. At other points, there appeared to be focal disintegration or ulceration of the wall with a "break-through" of this hyaline fibrinous material. Several such "ulcers" of the bronchiolar walls were seen.

The normal immediate peribronchiolar histologic architecture was completely disrupted by the following: (1) a tremendous influx of neutrophils and some eosinophils, even into the lymphoid aggregates (Figure 12); (2) necrosis of the hypertrophied lymphoid aggregates; (3) clusters of bacilli in and outside the blood vessels (Figure 13); (4) clumps of fibrin (Figure 14); and (5) hemorrhage and necrosis of blood vessel walls, the necrotizing vasculitis previously mentioned. At times this intense inflammatory, necrotizing, and hemorrhagic process of the immediate peribronchiolar elements was so intimately associated with necrosis of the bronchiolar wall that it was impossible to separate the two.

In the periphery of this lesion, the alveoli contained blood, edema fluid, and some fibrin in decreasing intensity. The lesion was limited at its outer periphery, but there was no evidence of a capsule or wall.

Mediastinum: The loose connective tissues surrounding the large vessels, the trachea and main bronchi, and the intrathoracic lymph nodes were at times infiltrated with granular, noncellular, eosinophilic material, some hemorrhage and a heterogeneous population of inflammatory cells, neutrophils, eosinophils and macrophages (Figures 19, 20 and 21). This lesion, except in the one instance previously noted (Monkey No. 927) was minimal and corresponded to the grossly observed lesion. The lesions were most intense around the intrathoracic nodes, which frequently were the site of massive necrosis and hemorrhage. Thus, for the purpose of this report, mediastinitis may be defined as edema and minimal hemorrhage of the mediastinal tissues, accompanied by, and probably secondary to, hemorrhage and necrosis of the intrathoracic lymph nodes.

Central Nervous System: The hemorrhagic meningitis, previously described in "B. Gross Pathology," was characterized by hemorrhage, masses of bacilli in and outside of blood vessels, and a marked neutrophilic and monocytic infiltration into the meninges (Figure 25). A concomitant lesion in the severe cases was the necrotizing lesion of the blood vessels previously mentioned (Figure 26). Occasionally these vessels were "cuffed" by bacilli.

No lesions were observed in the brain sections except for occasional, unimpressive, small perivascular hemorrhages in the cerebral cortex.

Liver: The lesions in this organ were identical with those seen in the inoculation infection in this host. Varying degrees of hepatocellular degeneration and necrosis were observed. Necrosis was focal and patchy, involving small groups of hepatic cells (Figure 23). Massive necrosis was not seen. Large numbers of bacilli were frequently observed in the sinusoids.

Endocrine Organs: As in the intradermal infection, the adrenal was the only gland frequently involved. The lesions were similar in that they were focal, singular or multiple, cortical hemorrhages with or without concomitant necrosis (Figure 22). Clusters of bacilli were present in the areas of cortical necrosis.

Thyroid: This organ was consistently spared as in the intradermal infection.

Genitourinary Tract: Significant lesions were observed only in the kidneys, as in the inoculation infection. In four instances, degenerative changes of tubular epithelium and tubular casts were seen. Casts with various characteristics were observed: amorphous, lightly basophilic, and proteinaceous; dull and hyaline-like; granular and brightly eosinophilic; and brightly eosinophilic, which looked like hemoglobin casts (Figure 29). The casts were observed primarily in distal convoluted and collecting tubules. In two instances the tubular epithelium throughout the cortex contained coarse eosinophilic granules or droplets (Figure 28) which varied in size. In one animal, presented an aerosol dose of 10^6 spores and dying on day 4 after exposure (Monkey No. 949), numerous bright eosinophilic casts resembling hemoglobin were observed in the loops of Henle and the distal convoluted tubules in the cortex (Figure 29). Most of these tubules were also dilated and were lined by flattened epithelium. Many of the collecting tubules in the medulla contained brightly eosinophilic and coarsely granular casts, as previously mentioned. The tubular epithelium in this case, both cortical and medullary, was generally intact, so that "lower nephron nephrosis" could not be seriously considered as a histologic diagnosis.

In the four cases with renal changes, casts were the principal lesion, the changes in tubular epithelium being relatively mild; large numbers of bacilli were present in the glomeruli and vessels. Focal cortical necrosis was rarely seen, and when seen did not involve glomeruli.

Bone Marrow: Focal necrosis was observed in the marrow of many animals. There was no generalized necrosis or significant myeloid depletion. Varying numbers of bacilli were recognized.

Cardiovascular System: Myocardial and subendocardial hemorrhages were seen in a few animals; in one instance there was accompanying focal necrosis.

Many of the capillaries and smaller blood vessels appeared to be virtually occluded with masses of bacilli. The principal vascular lesion has already been mentioned and is described fully elsewhere¹. In brief, it is essentially a necrotizing lesion of the walls of the small to medium sized vessels accompanied by intravascular thrombi or emboli (Figures 26, 27). The integrity of the vessel walls is completely destroyed and the walls appear as rings of non-cellular, amorphous, eosinophilic material in which no cellular detail can be recognized. These damaged vessels were always seen in areas of marked hemorrhage and necrosis, containing masses of bacilli, and were most prominent in the meninges and in lymph nodes. Although there may have been leukocytic infiltration into the site of hemorrhage and necrosis, there was none into

these vessel walls. Not all vessels were so involved. Special stains to demonstrate the elastica disclosed that even this structure did not escape the severe necrotizing process. The walls of the smaller vessels at times appeared thicker than normal, and their outlines were fuzzy rather than sharp.

Gastrointestinal Tract: As in the inoculation group, most of these animals had lesions of esophagostomiasis. These lesions as described by Ruch^{2/} are granulomatous lesions in the wall of the large intestine or in the mesentery. Nematodes of the *Strongyloides* genus were also frequently found in the mucosa accompanied by eosinophilic infiltrations and occasionally by superficial ulceration of the mucosa. Occasionally bacilli were associated with these lesions.

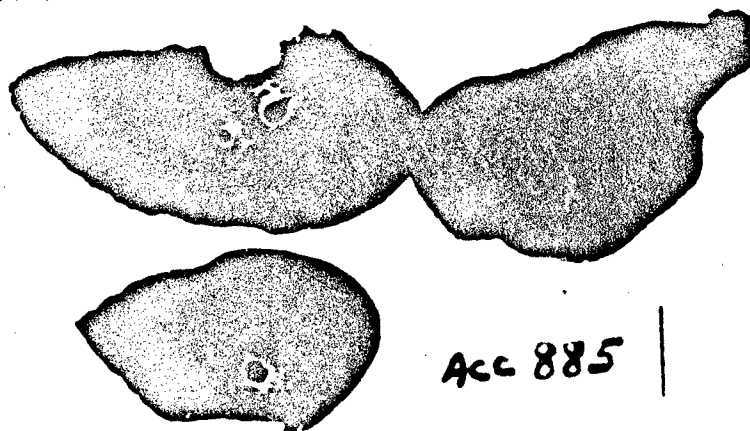
In one monkey (No. 676), presented with an aerosol dose of 50,000 spores and dead on day 7, there was an ulcer in the large intestine with an associated intense neutrophilic infiltration and a large mass of bacilli in the lesion. The mucosa was completely denuded, the muscularis mucosa ruptured, and the cellular infiltrate extended into the submucosa. Large numbers of bacilli were present in the mucosa adjacent to the ulcer. No parasite was found associated with this ulcer. A gastric ulcer was also seen in one animal (Monkey No. 774) that was exposed to an aerosol of 13,000 spores and died on day 6 with mediastinitis, hydrothorax, and severe hemorrhagic meningitis.

IV. DISCUSSION

The basic nature of the lesions seen and studied in this series of animals was no different from that seen in the terminal, intradermally inoculated animals^{1/}. Edema, hemorrhage, and necrosis, with varying degrees of leukocytic response in different tissues, were the cardinal pathologic lesions in both groups of animals. Necrosis of lymphatic tissues, liver, adrenals, and blood vessel walls, and occasionally focal necrosis of other tissues, such as renal, were observed in both groups. Hemorrhages were prominent in the lungs, lymphatic tissues, and adrenals in both groups, particularly in those spleens and lymph nodes which were severely depopulated and were virtually "bags of bacilli" with only the structural framework remaining. The lesion of necrotizing vasculitis was seen in these nodes. Hemorrhagic meningitis, accompanied by an intense leukocytic response and necrosis of vessel walls, occurred rarely in intradermally infected animals^{1/}. This lesion complex was observed much more frequently in the respiratory series of animals as seen in Table I.

Massive cellulitis developing at the site of inoculation in the intradermal animals was missing in this group. Instead there was "cellulitis" of the mediastinum.

The intrathoracic lymphadenopathy-mediastinitis-meningitis complex of lesions observed in this group of animals was observed and beautifully described by Greenfield^{6/} in his report on Woolsorter's Disease in man in 1881. At this time Greenfield established that Woolsorter's Disease was a form of anthrax blood poisoning and that "mediastinal cellulitis" in this disease may be secondary to an "intense lymphadenitis and hemorrhage of the bronchial glands." In these same studies he described hydrothorax, as observed in these animals. He further stated that "the virus entered at some point in the neighborhood of the mediastinal glands or draining through them by its lymphatics." Fraenkel^{12/} also



Acc 885

FIGURE 5. ACC. 885 - HEMORRHAGIC NODULE OF RESPIRATORY ANTHRAX IN M. MULATTA; "LUNG MITE - ANTHRAX" LESION.

FIGURE 6. ACC. 885 - "LUNG MITE - ANTHRAX" LESION ON SECTION - LIGHT CENTER & DARK PERIPHERY.

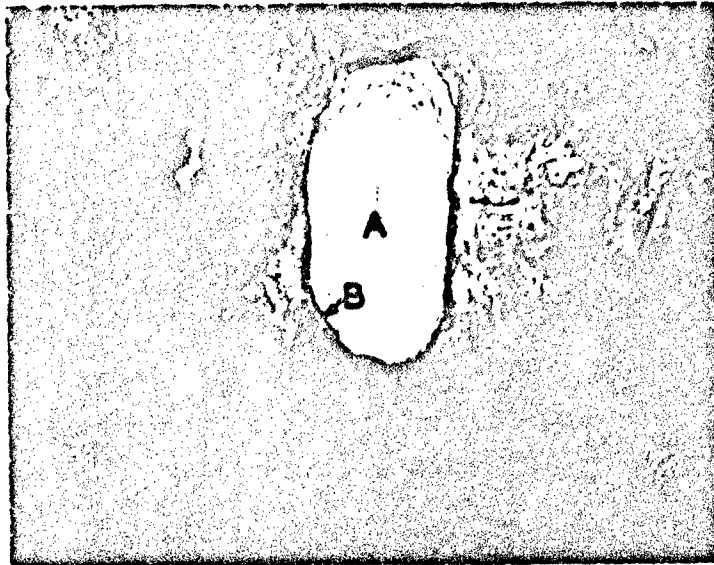


FIGURE 7. ACC. 885 - "LUNG MITE - ANTHRAX" LESION - DILATED BRONCHIOLE FILLED WITH FIBRIN-CONTAINING SERUM OR LYMPH (A), LINED BY A PSEUDOMEMBRANE (B), PERIBRONCHIOLAR LYMPHOID ELEMENTS ALTERED BY EDEMA & HEMORRHAGE WHICH EXTENDS INTO THE PULMONARY PARENCHYMA (C). X25.

FIGURE 8. ACC. 882 - "LUNG MITE - ANTHRAX" LESION WITH MITE (A) IN DILATED BRONCHIOLE, & WITH CONSIDERABLE HEMORRHAGE INTO PERIBRONCHIAL LYMPHATIC ELEMENTS (B). X25.

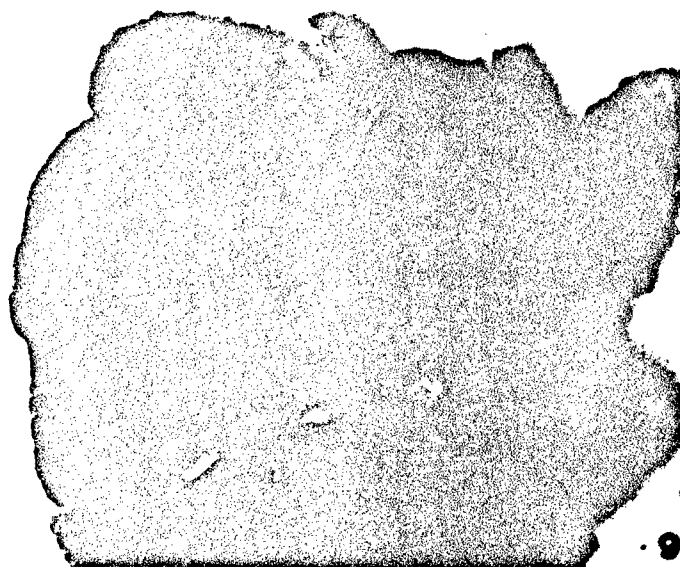
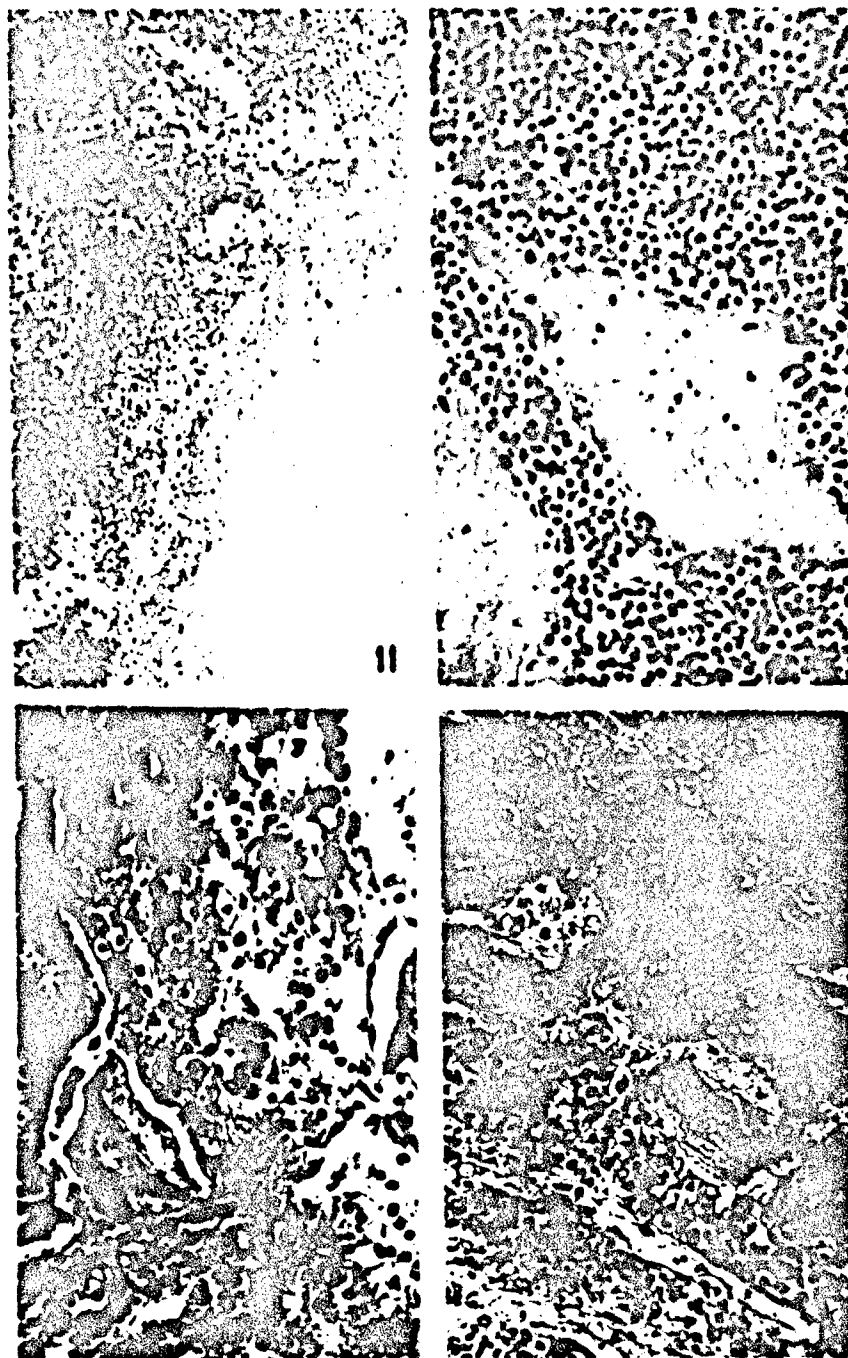


FIGURE 9. ACC. 884 - "LUNG MITE-ANTHRAX" LESIONS (A) WITH LIGHT CENTER & DARK PERIPHERY, & "NORMAL" MITE LESION (B).

FIGURE 10. ACC. 884 - SAME LESION - DILATED BRONCHIOLE CONTAINING LYMPH OR SERUM WITH FIBRIN (A), THE PARASITE (B), THE PSEUDOMEMBRANE LINING THE WALL OF THE BRONCHIOLE (C), & LYMPHOID ELEMENTS, ALTERED BY EDEMA, FIBRIN, & HEMORRHAGE (D). X25.



- FIGURE 11. ACC. 885 - "LUNG MITE - ANTHRAX" LESION - LACK OF EPITHELIAL LINING OF BRONCHIOLAR WALL, & THE PSEUDOMEMBRANE IN PLACE OF IT; LEUKOCYTIC INFILTRATION & HEMORRHAGE IN PERIBRONCHIOLAR LYMPHATICS. X60.
- FIGURE 12. ACC. 885 - SAME LESION - NEUTROPHILIC INFILTRATION INTO PERIBRONCHIOLAR LYMPHATIC ELEMENTS & DILATED LYMPHATIC VESSELS. X200.
- FIGURE 13. ACC. 885 - SAME LESION - MASSES OF BACILLI & SOME HEMORRHAGE. X200.
- FIGURE 14. ACC. 885 - SAME LESION - MASSES OF FIBRIN & CELLULAR INFILTRATE. X200.

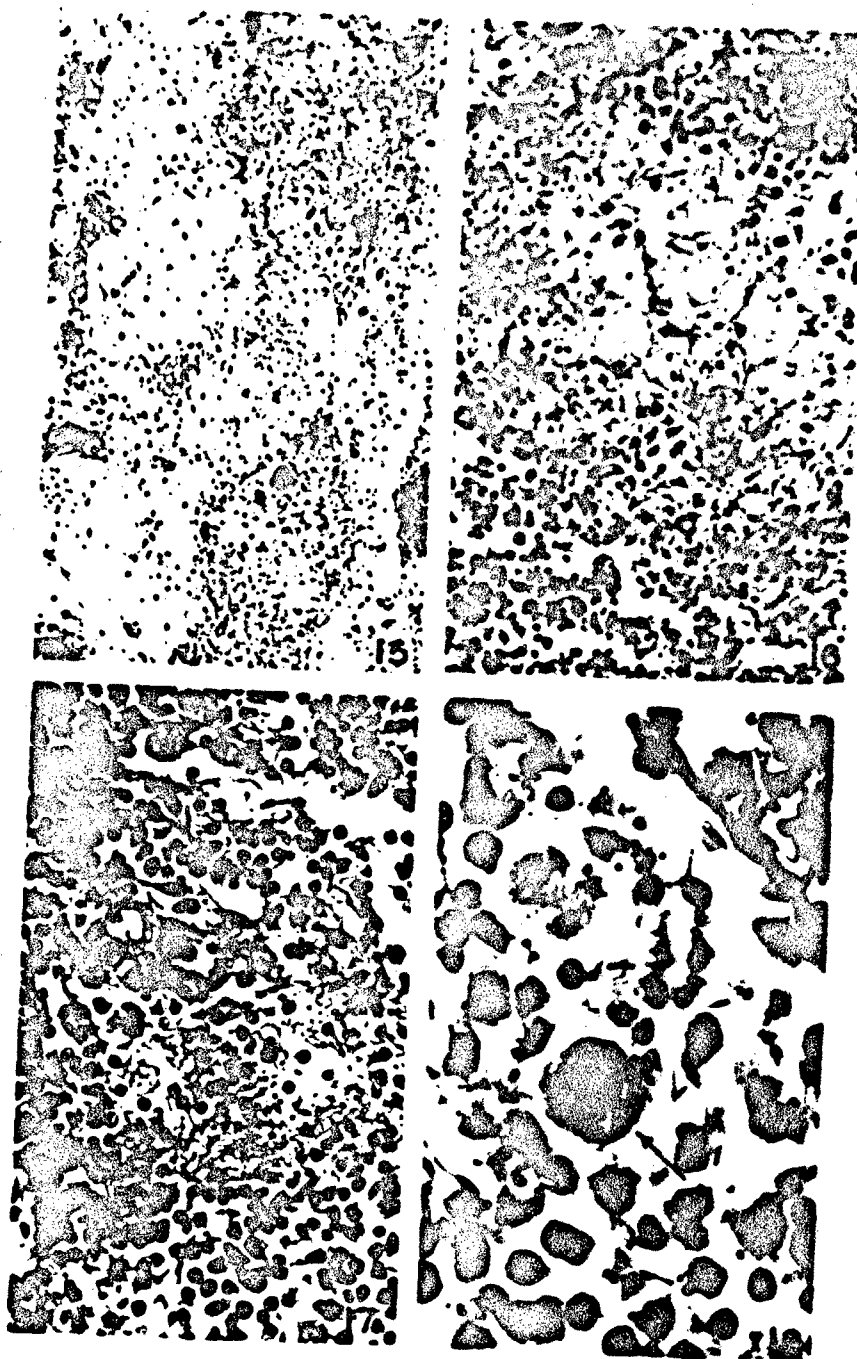


FIGURE 15. ACC. 885 - LYMPH NODE - MARKED LOSS OF LYMPHATIC ELEMENTS, EDEMA WITH SOME FIBRIN. X90.
 FIGURE 16. ACC. 885 - LYMPH NODE - MINIMAL HEMORRHAGE, MASSES OF FIBRIN. X180.
 FIGURE 17. ACC. 884 - LYMPH NODE - MASSES OF BACILLI. X340.
 FIGURE 18. ACC. 883 - LYMPH NODE - PHAGOCYTOSIS OF BACILLI. X600.



FIGURE 19. ACC. 885 - MEDIASTINUM. EDEMA (A), HEMORRHAGE (B), & FIBRIN (C).
(D) TRACHEA. X80.

FIGURE 20. ACC. 885 - MEDIASTINUM. EDEMA, FIBRIN, & HEMORRHAGE. X200.

FIGURE 21. ACC. 997 - MEDIASTINUM. MORE HEMORRHAGE THAN IN PREVIOUS LESION.
X25.

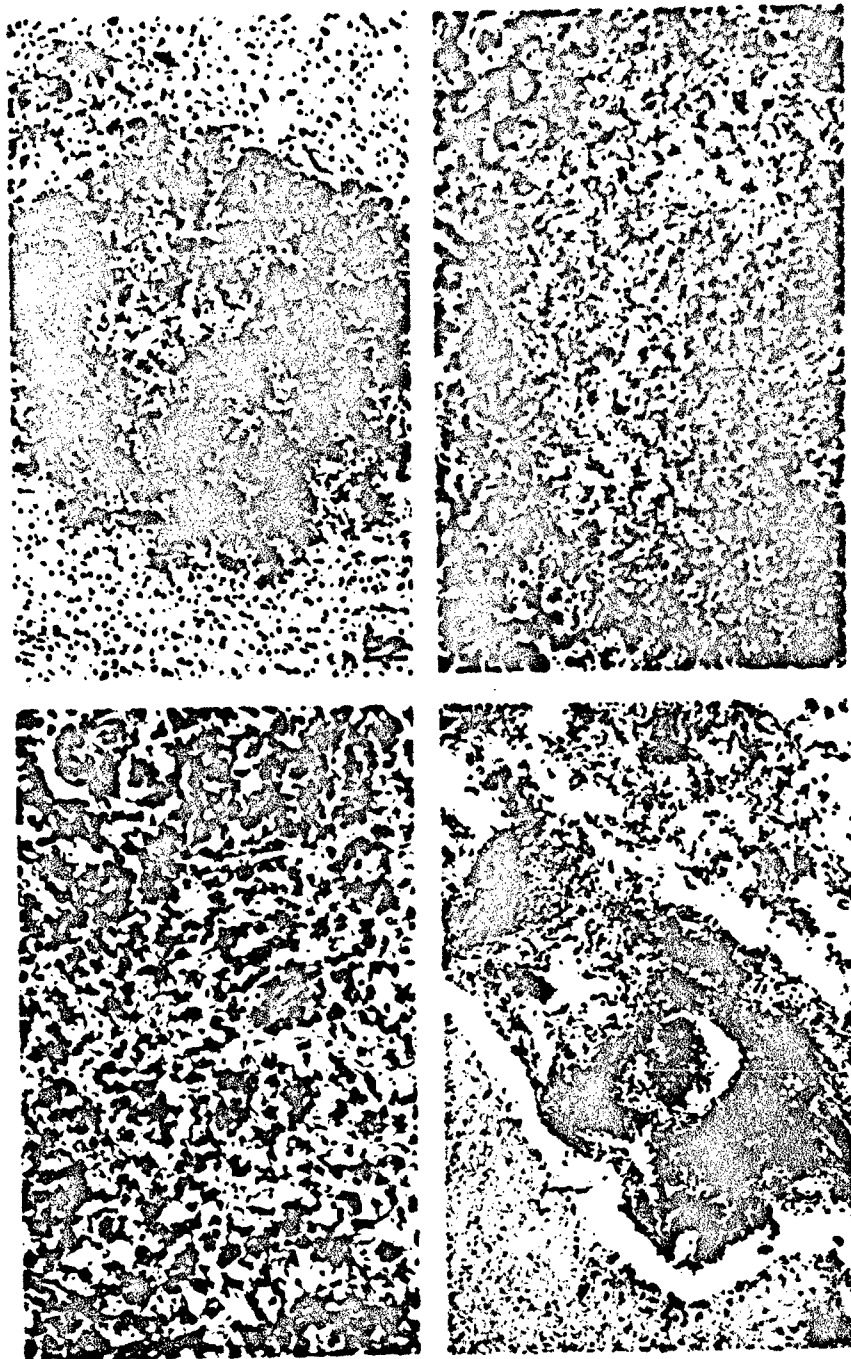


FIGURE 22. ACC. 917 - ADRENAL. HEMORRHAGE & NECROSIS. X90.

FIGURE 23. ACC. 753 - LIVER. FOCAL NECROSIS WITH MILD CELLULAR REACTION. X90

FIGURE 24. ACC. 917 - SPLEEN. SEVERE DEPOPULATION & MASSES OF BACILLI. X170

FIGURE 25. ACC. 884 - BRAIN. HEMORRHAGIC MENINGITIS. X80

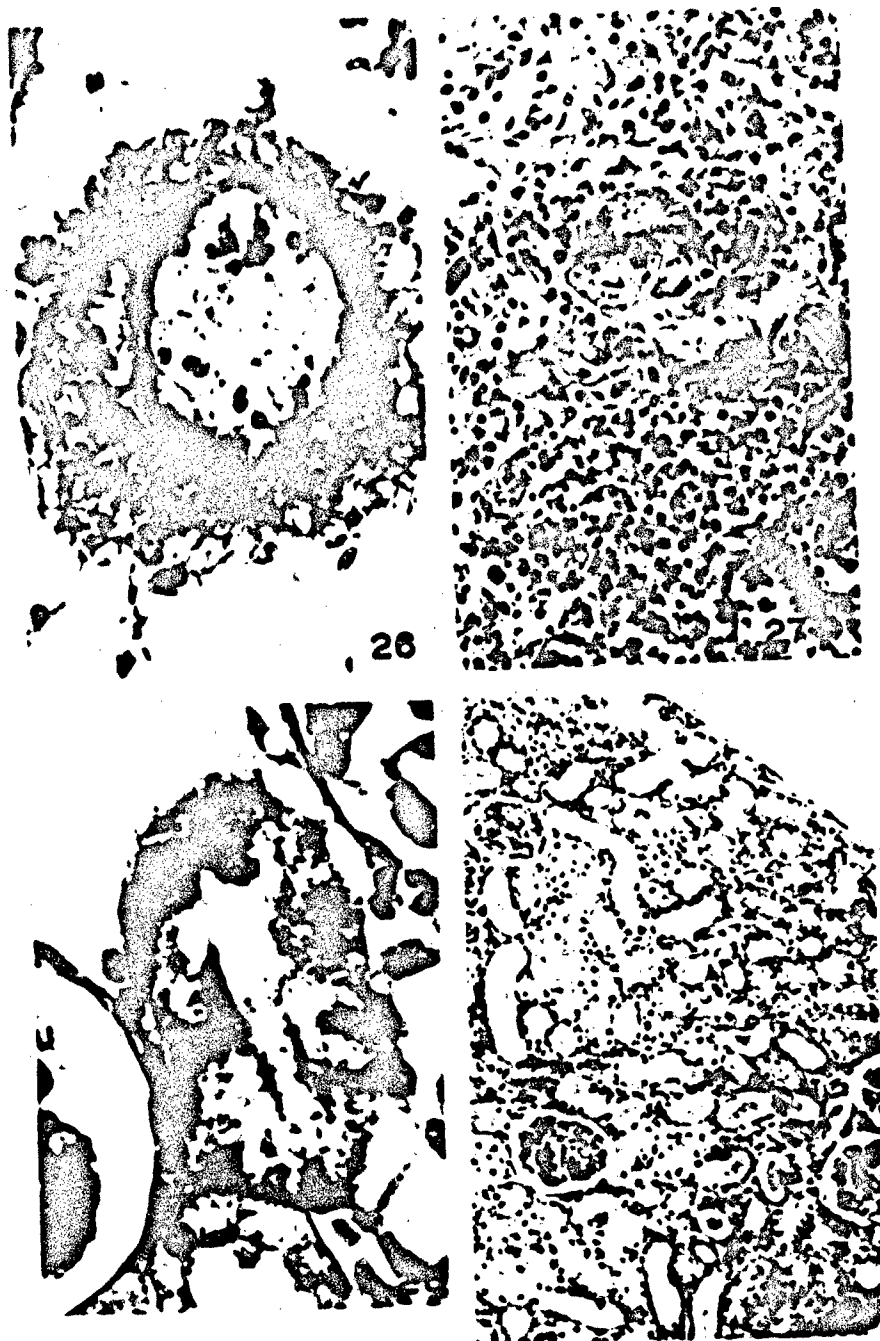


FIGURE 26. ACC. 884 - MENINGES. NECROTIZING VASCULITIS WITH BACILLARY CUFFING. X410.
 FIGURE 27. ACC. 885 - LYMPH NODE. NECROTIZING VASCULITIS. X180.
 FIGURE 28. ACC. 939 - KIDNEY. HYALINE DROPLETS IN EPITHELIAL CYTOPLASM. X570.
 FIGURE 29. ACC. 942 - KIDNEY. HEMOGLOBIN-LIKE CASTS IN DISTAL CONVOLUTED TUBULES & LOOPS. X94.

examined a number of cases of Woollsorter's disease and described a lesion in the walls of the trachea or bronchus similar to the typical "pustule."

Albrink^{13/} in his report on human inhalation anthrax observed hemorrhagic mediastinitis in 2 of 3 patients, but failed to find any tracheal or bronchial ulcerations or any primary anthrax pneumonia; he also described hydrothorax. Young *et al*^{2/}, Barnes^{4/}, Ross^{5/}, and Albrink^{3/} in recent years failed to find any evidence of primary anthrax lesions in the trachea or bronchi in experimental animals thus rendering further support to the concept of Eppinger^{14/} that spores in the normal animal are carried away from the lower reaches of the lung via the lymphatics. Ross^{5/} demonstrated in guinea pigs that spores are transported in macrophages via lymphatics to the regional lymph nodes, that is, the hilar and tracheobronchial lymph nodes where germination and multiplication of bacilli resulted in lesions similar to those described here. The failure to find "primary" lesions in the air passages of some of these animals, therefore, is not considered bizarre.

The observation that there may be minimal intrathoracic lymph node destruction, although bacteremia and marked splenic destruction exist, is indeed surprising and intriguing. Albrink^{13/} reported the same observation in his series of cases of human inhalation anthrax. This observation could be explained by a blood-borne dissemination directly from the lungs. Albrink postulated that "spores could lodge in a focus primarily ulcerated by an unrelated injurious agent and thus establish a superinfection with *B. anthracis*." Presumably then, direct blood dissemination of bacilli could occur. Young *et al*^{2/} quote the work of Velu who concluded that while it was difficult to infect experimental animals with spores alone via the respiratory tract, "anthrax infection of the lung" was easily established after the lung had been damaged by inhalation of chlorine gas.

The group of animals considered here does have built-in pre-existing damage due to the lung mite; the hemorrhagic nodules already described are believed to represent anthrax superinfection of these pre-existing lesions. It appears feasible that in some instances the primary infection and multiplication occur in the hypertrophied lymphoid elements of the parasite-infected and injured bronchioles. Dissemination then can have occurred directly via the blood stream, bypassing in part the draining mediastinal lymph nodes, but resulting in massive destruction of the spleen as a result of the large numbers of organisms present in that organ and a terminal massive bacteremia. Frank ulceration of the involved bronchiolar wall in these lesions was observed. This lesion has been described for coding purposes as a "parasitic bronchiolitis with bronchiectasis and a necrotizing superinfection with *B. anthracis*."

This is considered a lesion of special significance in the etiology and pathogenesis of respiratory anthrax infection in this host, and leads one to wonder about the significance of pre-existing lesions in lungs in certain cases of human inhalation anthrax. A predilection of *B. anthracis* for pre-existing lesions has been observed^{15/} in lesions of oesophagostomiasis in the monkey and caseous lymphadenitis in sheep. A caseous prescapular lymph node

was the only site from which B. anthracis was isolated upon sacrifice of a lamb following spontaneous recovery, in spite of a demonstrated bacteremia^{15/}.

Hemorrhagic meningitis in respiratory anthrax has been described in human cases by numerous authors^{6,13,16,17/}.

Cowdery^{18/} has described a case of "pulmonary anthrax" in man with bacilli-containing ulcers in the gastrointestinal tract. Only one intestinal ulcer and one gastric ulcer were observed in our group of animals. Several such lesions were observed in a group of monkeys receiving B. anthracis challenge by the respiratory route which were subsequently treated with antibiotics to alter the course of the disease^{15/}. The pathogenesis of this lesion in the monkey is not difficult to comprehend in view of the high incidence of *Strongyloides* infection in these animals, with resulting injury of the mucosa.

V. SUMMARY

The pathologic picture of respiratory anthrax in unmodified M. mulatta has been described and compared to that seen in this host infected intradermally. The basic nature of the lesions is the same in both groups of animals, in that they are primarily hemorrhagic and necrotizing. The distribution of lesions was somewhat different; in the respiratory group there was high incidence of mediastinitis in lieu of the cellulitis seen in intradermally infected animals. Associated with this mediastinitis there was also a high incidence of hemorrhagic meningitis, hemorrhagic pulmonary lesions and intrathoracic lymphadenopathy. The incidence of some of the lesions described appeared to be dose-dependent.

The significance of the lung mite lesions in the pathogenesis of respiratory anthrax in this host was discussed, and the predilection of B. anthracis for pre-existing lesions was brought out. The possibility of direct blood dissemination of the organism from a "super-infection" of a pre-existing lung lesion, bypassing the draining lymph nodes, was discussed.

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STUDIES ON BACILLUS ANTHRACIS

PART 5

PATHOGENESIS OF RESPIRATORY ANTHRAX IN MACACA MULATTA, SERIALY SACRIFICED
(Berdjis, Gleiser, Hartman, Kuehne and Gochenour)I. INTRODUCTION

Previous reports have endeavored to describe the histopathology and pathogenesis of anthrax in Macaca mulatta monkeys challenged by the intradermal^{1/} and respiratory routes^{2/}.

Literature gives many references of respiratory anthrax in experimental animals^{3-8/} and in man^{9-13/}, in which attempts were made to determine the site of germination of Bacillus anthracis spores and establish the route of dissemination. The pathogenesis of this infection is still obscure.

This paper presents additional data obtained by serial sacrifice in an attempt to clarify this situation and to detect the initial lesion responsible for respiratory anthrax before overwhelming septicemia is established.

II. MATERIALS AND METHODS

Twenty-two young, immature, healthy M. mulatta monkeys were used in these experiments.

Two respiratory exposures were made with a modified Henderson dynamic aerosol generating device. Two animals were exposed simultaneously to each cloud generated. Concentration and virulence of the spore suspension aerosolized were determined by plate counts on nutrient agar and subcutaneous titration in guinea pigs. The spore suspension used was Vollum-189 strain of B. anthracis, stored in a concentration of 5×10^{10} spores/ml at 5°C. The appropriate dilutions of this material were heat-shocked 48 hours prior to use. Respiratory doses presented were estimated by assay of impinger recoveries for each exposure port.

In the first experiment 14 monkeys were exposed to seven clouds which contained presented doses of approximately 2×10^5 spores (Low Dose). Temperatures were taken twice a day and roentgenograms were obtained daily using a 1 MEV machine. Two monkeys were sacrificed per day, days 1 through 6, except day 3 which had four animals. At autopsy quantitative bacteriology was done on hilar and tracheobronchial lymph nodes, lung, spleen and blood. Blood was also inoculated into diphasic media.

In the second experiment eight monkeys were exposed to four clouds which contained presented doses of approximately 2×10^6 spores (High Dose). Temperatures and x-rays were obtained daily. Two monkeys were sacrificed per day for three days starting on day 1. Blood cultures were made quantitatively at time of sacrifice. Two died on day 2 of anthrax; they are not included.

All animals were autopsied routinely and representative sections of every organ were fixed in 10 per cent formalin. Routine histologic procedures were used. Hematoxylin and eosin (H&E) stain was regularly employed. Periodic acid Schiff (PAS)^{14/}, with and without diastase digestion, and Brown and Brenn (B&B), for bacilli, were used frequently. A modified acid-fast stain, after Rosa^{1/}, and a modified Weigert's stain were also employed for detection of spores in tissue sections. Blood smears were also examined at time of autopsy.

Technique for lung examination. Both lungs and trachea were fixed in formalin. Each lung was then sliced by transverse sections at regular intervals. In most animals every slice was embedded in paraffin for microscopic examination; in the others only representative sections were so handled. In order to demonstrate a possible solitary lesion, serial sections were made in each suspicious portion and every fifth to tenth section was examined.

III. RESULTS

A. CLINICAL OBSERVATIONS

No specific clinical observations were noted during this study with the exception of occasionally slightly elevated temperatures (Table I). Daily roentgenograms revealed no demonstrable lesions in lungs and mediastinum. Tables II and III summarize bacteriologic findings.

B. GROSS PATHOLOGY

A summary of the gross changes (Table I) suggests a dose-time-relationship in experimental respiratory anthrax. As early as day 2, lesions were found in the respiratory system and its tributary lymph nodes. These were increased in intensity on day 3. At the High Dose there were no animals available to sacrifice on day 4 so findings for that day are not known. At this Low Dose gross examination revealed no consistent changes on days 4 through 6.

No meningitis or substantial mediastinitis were observed in these animals differing somewhat from the observations in terminal respiratory anthrax^{2/}. There was no evidence of tracheal or bronchial ulcers, described by Fraenkel^{11/} as the site of inoculation of "primary anthrax lesion."

Special consideration is reserved for the "pulmonary mite lesion," described by Innes et al^{15/} and noted in an earlier report^{2/} as an almost constant finding in the lungs of M. mulatta. In connection with this lesion, "hemorrhagic parasitic nodules"^{2/} were observed on day 3 in both Low and High Dose animals.

C. MICROSCOPIC PATHOLOGY

1. Low Dose

Day 1 (2 monkeys):

Lymphatic System: The lymph nodes showed minimal edema with dilated lymph channels without significant pathologic changes. The architectural

TABLE I. MAXIMUM TEMPERATURES AND GROSS PATHOLOGY OF MONKEYS INFECTED WITH ANTHRAX BY THE AEROSOL ROUTE AND SERIALY SACRIFICED

DAY Sacrifice	DOSES/ fice	MONKEY Acc. No.	MAXIMUM TEMP. °F	GROSS PATHOLOGY		
				Lymphadenopathy ^b /	Respiratory System	Other
1	Low	912	NC ^c	NR ^d	NR	NR
		913	N	NR	NR	NR
	High	893	104.0	NR	NR	NR
		894	104.2	NR	NR	NR
2	Low	914	104.0	Minimal	NR	Mediastinitis, edema, minimal
		915	N	Moderate	Hemorrhage	Mediastinitis, edema, moderate
	High	896	N	Moderate	NR	Mediastinitis, edema
		898	N	Minimal	NR	NR
	Low	918	104.0	NR	NR	NR
		916	105.2	Minimal	NR	NR
917		105.4	Minimal	Hemorrhagic nodule	Adrenal hemor- rhage	
3	High	919	104.2	Minimal	NR	Adrenal hemor- rhage
		901	N	Minimal	NR	Meningeal in- jection
	902	104.2	Mild	Hemorrhagic nodule	Mediastinitis, edema	
	4	Low	920	104.4	NR	NR
921			N	NR	NR	NR
5	Low	922	N	NR	Hemorrhage	Mediastinitis, edema, minimal
		923	N	NR	NR	NR
6	Low	924	N	NR	NR	NR
		925	104.4	NR	NR	NR

a. Low dose: 1×10^5 - 2×10^5 ; high dose: 1×10^6 - 2×10^6 .

b. Hilar and tracheobronchial nodes.

c. N: Normal temperature.

d. NR: Not remarkable.

TABLE II. RESULTS OF BACTERIOLOGIC STUDIES AT AUTOPSY OF MONKEYS
INFECTED WITH ANTHRAX: LOW DOSE

DAY (Sacrifice)	MONKEY Acc. No.	CULTURE					SMEAR	
		Blood	Lung	Lymph Node		Spleen	Blood	Spleen
				Hilar	Tracheobronchial			
1	912	-	+	-	-	-	-	-
	913	-	+	-	-	-	-	-
2	914	+	+	+	+	+	-	-
	915	+	+	+	+	+	-	+
3	918	-	+	-	-	-	-	-
	916	+	+	+	+	+	+	+
	917						+	
	919						+	
4	920	+	+	+	+	+	-	-
	921	-	+	-	-	-	-	-
5	922	-	+	+	-	-	-	-
	923	-	+	-	-	-	-	-
6	924	-	+	-	-	-	-	-
	925	-	+	-	-	-	-	-

TABLE III. RESULTS OF BACTERIOLOGIC STUDIES AT AUTOPSY OF
MONKEYS INFECTED WITH ANTHRAX: HIGH DOSE

DAY (Sacrifice)	MONKEY Acc. No.	BLOOD CULTURE	SMEAR	
			Blood	Spleen
1	893	-	-	
	894	-		
2	896	+		
	898	+	-	
3	901	-	-	
	902	+	-	+

pattern was not disturbed; no bacilli were detectable within the parenchyma. Minimal changes were found in occasional lymph nodes especially at the level of germinal centers, with no clearcut relationship to the present experimental conditions. Similarly, the spleen showed only moderate congestion with increased cellular debris and macrophages. No bacilli were detectable.

Other organs: The only detectable lesions elsewhere were occasional small localized subpleural hemorrhages, perhaps related to mite lesions, and congestion of the organs. No bacilli were detectable. Hepatic cells of the centrilobular portion of liver were rich in diastase-digestible PAS-positive material and multiple small droplets of fat (Figure 1); no other morphologic changes were observed. The Kupffer cells showed minimal non-glycogenic PAS-positive material.

Day 2 (2 monkeys):

Lymphatic System: All lymph nodes showed some edema and congestion with fairly well-preserved architectural pattern. There was no significant hemorrhage or necrosis. Occasional bacilli were detectable only in tracheobronchial lymph nodes. There were increased numbers of macrophages and cellular debris with apparent erythrophagocytosis. Some of the dilated lymph channels contained homogeneous, eosinophilic pale fluid with or without inflammatory cells, but no bacilli. In the spleen the architectural framework was not destroyed. The parenchyma was markedly congested; there were small foci of hemorrhage in isolated and slightly depleted follicles. Cellular debris and macrophages were moderately increased. Occasional bacilli were visible in the parenchyma.

Respiratory System: These animals showed no pulmonary lesions attributable to anthrax, even though parasitic lesions were present. Minimal edema, focal extravasation, and a few macrophages were found with the alveoli. No bacilli were detectable in multiple sections of lungs.

Liver: Findings were similar to those of day 1. No bacilli were detectable.

Other organs: Minimal to moderate congestion was noted. All were devoid of bacilli.

Day 3 (4 monkeys):

Lymphatic System: All stages were observed, from unharmed lymph nodes in a non-septicemic animal (Acc. 918) to partly hemorrhagic and necrotic nodes in septicemic monkeys (Acc. 916 and 919). The tracheobronchial and hilar nodes were the most severely damaged. In another septicemic animal (Acc. 917) the architectural pattern showed no disorder nor was there lysis. The lymphatic tissue and lymph channels, although dilated, contained very few bacilli (Figure 2). This observation is contrasted with the presence of masses of bacilli within the blood vessels in splenic parenchyma ("bag of bacilli"). Spleens showed none to marked disorder and lysis.

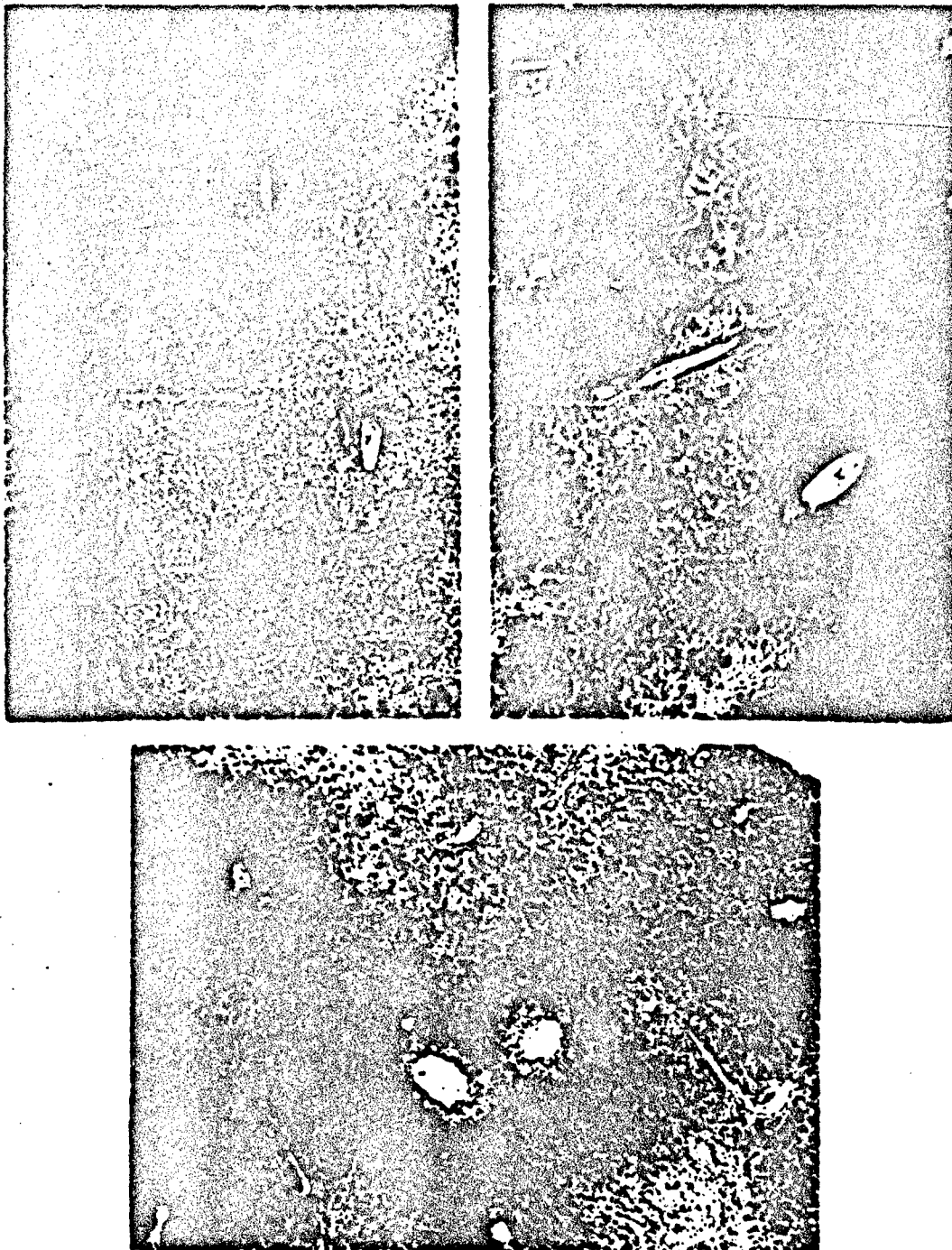


FIGURE 1. LIVER PAS, X51 (A) CONTROL (ACC. 1078). (B) DAY 1 (ACC. 912) CENTRIOCITULAR ORIENTATION OF NUMEROUS DROPLETS & DUST-LIKE DIASTASE DIGESTIBLE PAS-POSITIVE MATERIAL (DARK SPOTS OR BLACK DOTS). (C) DAY 2 (ACC. 913) INCREASED ACCUMULATION WITH MORE ORIENTATION.

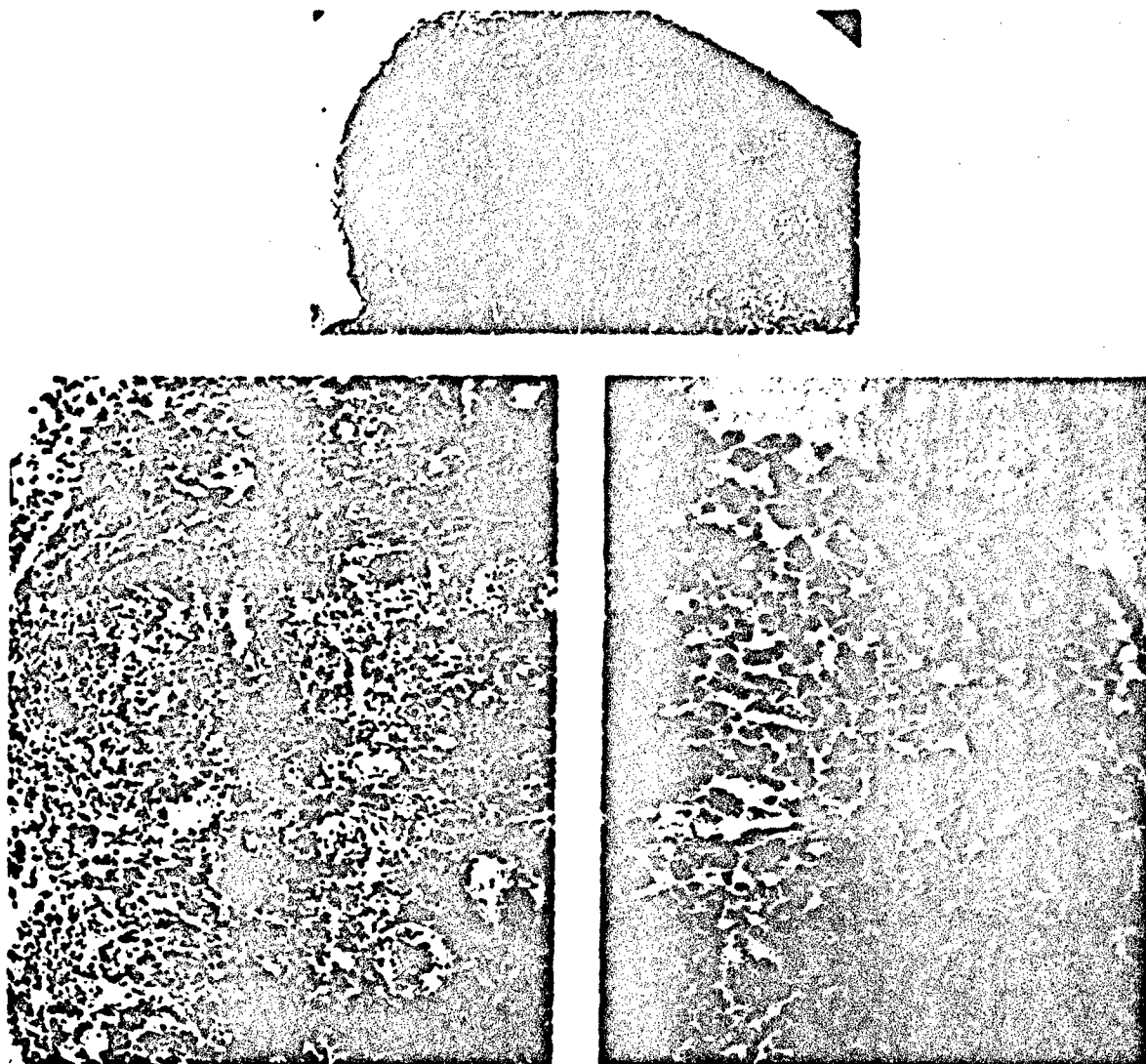


FIGURE 2: TRACHEOBRONCHIAL LYMPH NODE, LOW DOSE, DAY 3 (ACC. 917).
(A) FAIRLY WELL-PRESERVED ARCHITECTURAL PATTERN; NO SIGNIFICANT
HEMORRHAGE OR NECROSIS. H&E X35. (B) & (C) SAME, MANY BACILLI
IN BLOOD VESSELS & NONE IN LYMPH CHANNELS & SUBCAPSULAR
SINUSES. B&B. (B) X115 (C) X230.

Respiratory System: "Specific pulmonary lesions"^{2/}, consisting of "superinfection" of anthrax on a pre-existing mite lesion, were seen. These lesions consisted of necrotizing bronchiolitis rich in bacilli with concomitant bronchiolectasis. The lumen of such a bronchiole was usually enlarged and filled with pale pinkish fluid containing numerous bacilli and a few inflammatory and desquamated cells (Figure 3C). The bronchiolar space also contained fibrin strands and a thick layer of hyalinized and necrotic dense material, rich in bacilli. The adjacent pulmonary parenchyma revealed edema, hemorrhage, varying numbers of bacilli and marked inflammatory infiltrates which were both acute (anthrax) and chronic (parasitic). Remaining lung parenchyma and other mite lesions were noncontributory and unremarkable, even when the bacilli were present in alveolar capillaries (Figure 3.A, B, and D).

Other organs: In two of four animals the adrenal glands showed focal hemorrhage and necrosis with congestion of the cortex. Other organs were not involved. The hepatic parenchyma was inconspicuous; however, a PAS stain revealed an increase in non-glycogenic material in prominent Kupffer cells. These cells contained bacilli (Figure 4). A fat stain revealed fat droplets in hepatic cells, especially in the centrolobular portion.

Day 4 (2 monkeys):

Lymphatic System: Lymph nodes and spleen showed minimal edema and moderate congestion with occasional small areas of hemorrhage. No necrosis was detectable. In one animal occasional bacilli were seen in some lymph nodes, none, in the spleen.

Respiratory System: Although some parasitic lesions were present, no significant pathologic changes were observed in lung parenchyma. No bacilli were seen.

Other organs were unremarkable and unmodified.

Day 5 (2 monkeys):

Multiple foci of pulmonary hemorrhage in one animal and moderate edema and congestion of lymphoid organs were the only findings. No bacilli were seen. Other organs were unmodified.

Day 6 (2 monkeys):

No significant pathologic changes attributable to anthrax were seen. No bacilli were observed.

2. High Dose

Day 1 (2 monkeys):

No significant pathologic changes nor bacilli were seen in any organ.

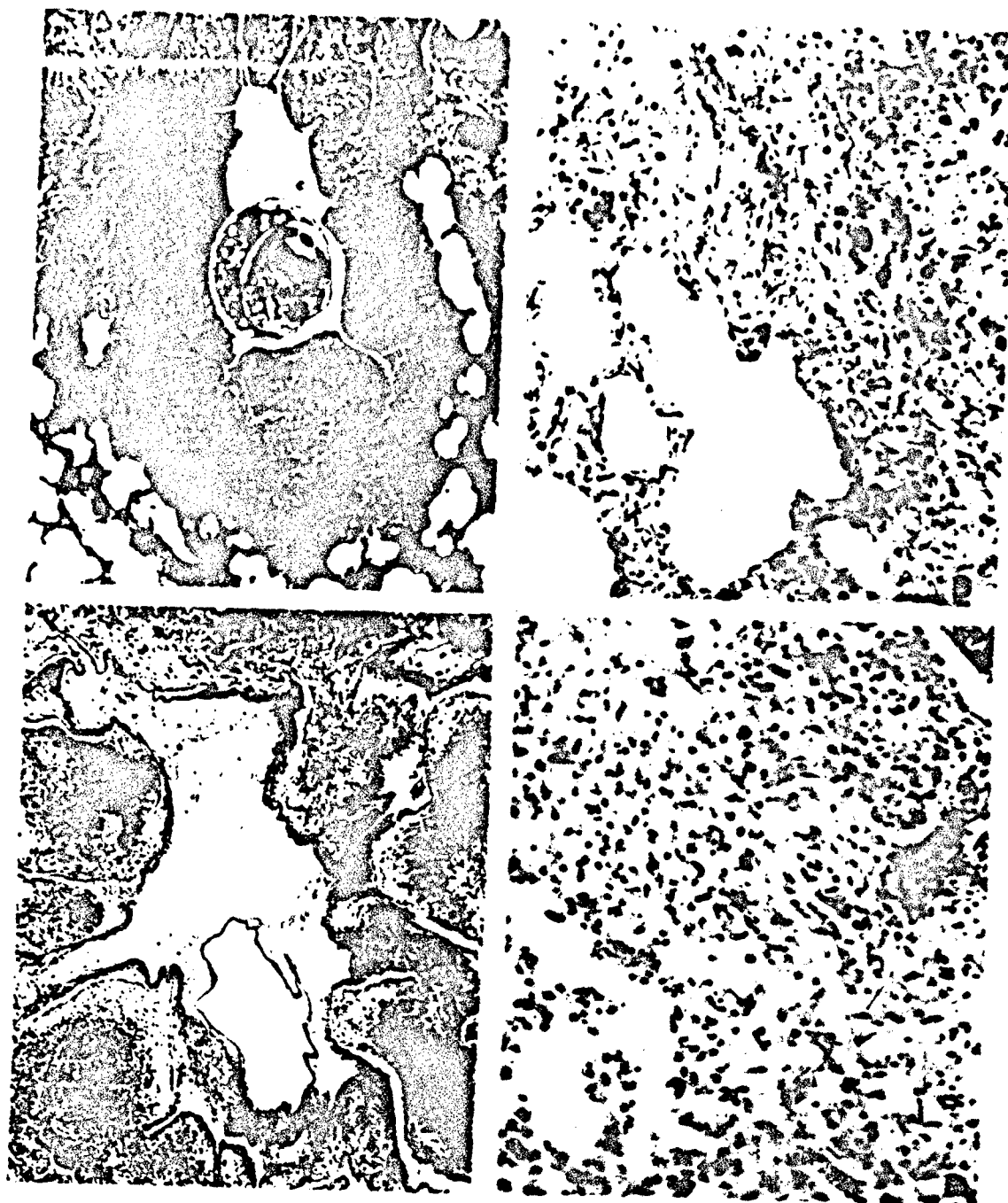


FIGURE 3 LUNG, LOW DOSE, DAY 3. (A) COMMON PULMONARY MITE LESION APPEARING AS CHRONIC BRONCHIOLITIS UNAFFECTED BY ANTHRAX EVEN THOUGH ALVEOLAR SYSTEM EXHIBITS MANY BACILLI AS SHOWN IN "B". (ACC. 918) H&E X48. (B) SAME, SHOWING NUMEROUS BACILLI IN ALVEOLAR WALL & NONE AT EDGE OF PARASITIC LESION (SHOWN IN UPPER RIGHT CORNER). (ACC. 918) B&B X220. (C) "SPECIFIC PULMONARY LESION," NECROTIZING & ULCERATIVE BRONCHIOLITIS WITH BRONCHIOLECTASIS; MARKED DESTRUCTION OF NEIGHBORING TISSUES BY BACILLARY INVASION. (ACC. 918) H&E X48. (D) EARLY INVASION OF NEIGHBORING TISSUES ADJACENT TO MITE LESION IN RELATIVELY UNHARMED ALVEOLAR SYSTEM. (ACC. 918) B&B X220.



FIGURE 4. LIVER, LOW DOSE, DAY 3 (ACC.917). PROMINENT KUPFFER CELLS PHAGOCYTIZING NUMEROUS BACILLI & EXHIBITING MANY NONGLYCOGENIC PAS-POSITIVE PARTICLES. PAS X848.

Day 2 (2 monkeys):

Lymphatic System: Lymph nodes were damaged slightly more than those of Low Dose animals. Although a number of nodes were congested and edematous, the architectural pattern was well-preserved. Occasionally there were dilated sinuses, increased cellular debris and macrophages, and varying degrees of depletion, especially in tracheobronchial nodes. These revealed hemorrhage and necrosis with numerous bacilli. The architectural pattern of the splenic parenchyma was markedly disturbed by diffuse necrosis. Most of the follicles were depopulated and exhibited necrotic centers with minimal peripheral hemorrhage. Bacilli were especially numerous in the necrotic areas.

Respiratory System: Necrotizing ulcerative bronchiolitis ("specific pulmonary lesion") was observed in one animal (Acc. 898). Figure 5 illustrates this finding in step sections at four levels. The neighboring tissue was involved and disclosed myriads of bacilli, numerous inflammatory cells and diffuse hemorrhage. This resulted in complete destruction of underlying parenchyma within the focus of involvement. Elsewhere the pulmonary parenchyma was unremarkable.

Liver: Extensive central and midzonal early hepatocellular degeneration and marked cloudy swelling were seen. No frank necrosis or hemorrhage were present. The cloudy swelling was accompanied by multiple small droplets of fat, especially at the centrilobular portion. The sinusoids were dilated and contained varying numbers of bacilli. Prominent Kupffer cells also contained many bacilli. There was also an increase in the PAS-positive material in the Kupffer cells.

Other organs: Apart from small, focal hemorrhages in the adrenal gland, no other significant pathologic changes were seen.

Day 3 (2 monkeys):

Lymphatic System: Most lymph nodes and spleens revealed no disturbance of architectural pattern, although they contained bacilli. Varying amounts of edema, congestion, erythrophagocytosis and cellular debris were observed in all lymph nodes. Only the tracheobronchial nodes revealed moderate to marked destruction of parenchyma by hemorrhage with or without necrosis.

Respiratory System: One "Specific pulmonary lesion" was found in the lungs of each animal.

Liver: There was a moderate amount of hepatocellular degeneration and cloudy swelling, accompanied in one animal by small foci of necrosis. No bacilli were recognized and no significant inflammatory response was observed. There were prominent phagocytic Kupffer cells containing numerous bacilli and non-glycogenic PAS-positive material.

Other organs: Occasional small foci of necrosis with or without hemorrhage were found in adrenal gland and bone marrow. The central nervous system showed edema, congestion, and an early meningeal reaction.

3. Table IV summarizes the significant microscopic findings.

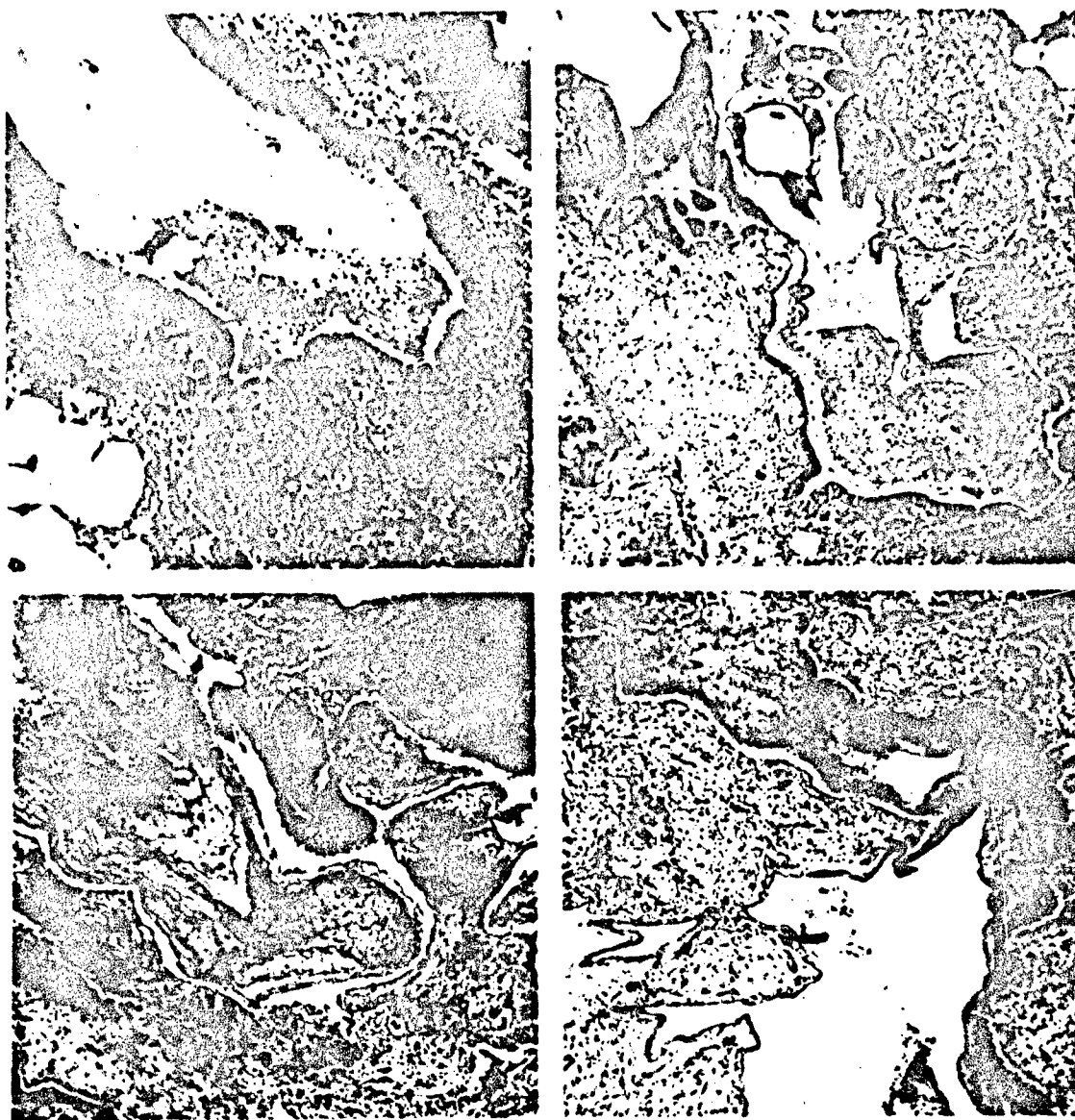


FIGURE 5. LUNG, HIGH DOSE, DAY 2 (ACC. 698). "SPECIFIC PULMONARY LESION" (STEP SECTIONS). H&E X57 (A) SMALL, LOCALIZED BRONCHIOLAR ULCERATION; MARKED PERIBRONCHIAL INFLAMMATION & ENLARGED LUMEN CONTAINING FIBRINOUS MATERIAL & DESQUAMATIVE & INFLAMMATORY CELLS WITH SOME BACILLI. (B) SECTION 20: MORE EXTENSIVE NECROTIZING ULCERATION, PROMINENT INFLAMMATORY REACTION & NUMEROUS BACILLI; SECTION OF MITE IN LUMEN. (C) SECTION 65: MYRIADS OF BACILLI IN LUMEN & PERIBRONCHIAL AREA; EPITHELIAL LINING ENTIRELY DESTROYED BY SUPERINFECTION (D) SECTION 90: MORE DESTRUCTION & MORE EXTENSION SEVERAL ALVEOLAR BRONCHIOLES ARE INTERCOMMUNICATING; ALL EC ALLY DAMAGED.

TABLE IV. SUMMARY OF MICROSCOPIC PATHOLOGY OF SERIALY SACRIFICED MONKEYS INFECTED WITH ANTHRAX BY THE RESPIRATORY ROUTE

ORGANS	DAY SACRI- FICES/	DILATED LYMPHATIC CHANNELS				EDEMA		HEMORRHAGE		NECROSIS		INFLAMMATORY INFILTRATE	
		BACILLI		L		L		L		L		L	
		L	H ^a /	L	H	L	H	L	H	L	H	L	H
Lung	1	-	-	±	±	±	±	Focal, minimal	Focal, minimal	-	-	-	-
	2	-	+	+	+	+	+	(+)c/ (+)	-	+d/	+	+	(+)
	3	+	+	+	+	+	+	+	+d/	+d/	+	+	+
Lymph Nodes	1	-	-	+	+	±	±	Focal minimal	Minimal	-	-	-	-
	2	(+)	+	+	+	+	+	- (+)	- (+)	±	±	+	+
	3	+	+	+	+	+	+	(+) (+)	(+) (+)	(+)	(+)	+	+
Spleen	1	-	-	±	±	±	±	-	-	-	-	-	-
	2	(+)	+	±	±	+	+	(+) +	-	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+
Liver	1	-	-	-	±	-	±	Hepatocellular degeneration	-	-	-	-	-
	2	-	+	±	+	±	+	±	-	-	-	-	-
	3	+	-	+	+	+	+	+	-	+	±	±	±
Adrenal	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	+	-	-	-	-	(+) +	(+)	+	+	-	-
	3	+	+	-	-	-	-	+	+	+	+	-	-

a. The findings on days 4, 5 & 6 are inconsistent and inconclusive.

b. L = low doses; H = high doses.

c. () = focal.

d. Necrotizing & ulcerative bronchiolitis with bronchiolectasis.

IV. DISCUSSION

The findings for day 1, both for High and Low Doses, were inconstant. Whether the changes, such as increased cellular debris, minimal hemorrhage, and necrosis of an occasional lymph node, were due to experimental anthrax could not be proved. There was an accumulation of PAS-positive material and fat particles in liver with a centrilobular orientation. Inasmuch as bacilli were not demonstrable and blood cultures were negative, it was not clear whether these changes were related to anthrax infection. This PAS-positivity, fat accumulation and hepatocellular degeneration, even with no bacilli detectable, warrants further investigation.

The first overt anthrax lesion appeared in the lung on day 2 in High Dose monkeys, with nothing significant at Low Dose. Despite positive blood cultures in both groups, no bacilli were seen in lungs of Low Dose monkeys and only a few in tracheobronchial lymph nodes. In High Dose animals bacilli were conspicuous and numerous at these sites. One is thus forced to conclude that entry of B. anthracis into the blood stream may precede the appearance of any recognizable lesion in monkey lungs.

Yet M. mulatta frequently does develop a lesion in pulmonary parenchyma, a necrotizing bronchiolitis superinfecting on a pre-existing parasitic involvement. Selected step sections of suspicious areas of lung revealed only one such lesion rich in bacilli; all the others were virtually devoid of organisms although the animal was septicemic. This combination strongly suggests that the lesion was the result of organisms being implanted directly by inhalation, followed by germination and multiplication.

These two observations can be reconciled at the present time only by assuming that organisms can reach the hilar nodes and/or the blood stream from the monkey lung in a manner comparable to that described by Rosa^{7/} for the guinea pig. The present studies do not confirm or deny the existence of such a mechanism. This initial process may continue as an overt septicemia or, as described by Albrink^{8/} in the chimpanzee, may be controlled by the host. In addition, in M. mulatta, organisms impinging on damaged lungs may germinate at the site and thus produce a continuing flow of organisms, either to lymph nodes or directly into the blood stream. The result is to place this susceptible animal in double jeopardy with either or both routes operative. In theory, in "resistant" animals the local lung lesion would be of considerable importance, thus insuring a continuing source for blood stream or lymph node involvement. Findings in one human case at autopsy^{13/} conform to this hypothesis.

Perhaps the injured bronchiole may serve to facilitate the initial "direct" entry of B. anthracis, being somewhat comparable to the additive effect described by Velu et al^{3,4/} for chlorine as a "pre-existing" injurious agent.

V. SUMMARY

One of the sites of initiation of infection in respiratory anthrax in M. mulatta is the bronchiolar wall providing a pre-existing parasitic lesion is present. When anthrax is superimposed, there results a necrotizing and

ulcerative bronchiolitis with focal bronchiolectasis, the "specific pulmonary lesion."

The mechanisms by which anthrax becomes disseminated are discussed. Evidence is presented that dissemination may result either by lymphatic drainage or by the blood stream.

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STUDIES ON BACILLUS ANTHRACIS

PART 6

PATHOLOGY OF SUBCUTANEOUSLY INDUCED ANTHRAX IN CHIMPANZEES
(Berdjis, Gleiser, Gochenour and Hartman)I. INTRODUCTION

The detailed pathologic observations and pathogenesis of experimental intradermal anthrax in Macaca mulatta were presented earlier^{1/}. Although numerous reports have been published on experimental anthrax in a variety of laboratory animals, no observations are available in the chimpanzee infected by the cutaneous route. Albrink and Goodlow^{2/} reported on four chimpanzees infected by the respiratory route.

The purpose of this paper is to portray the pathogenesis and describe the histopathologic changes occurring in chimpanzees inoculated subcutaneously with spores of Bacillus anthracis and to compare these findings with those found previously in M. mulatta.

II. MATERIALS AND METHODS

Eleven, young, adult, healthy chimpanzees*, seven of which were females, were inoculated with the following doses of B. anthracis spores: 500, 5000, 10,000 and 50,000. The dose distribution data is presented in Table I.

TABLE I. DISTRIBUTION OF CHIMPANZEES BY INFECTING DOSES

GROUP	DOSE (NO. OF SPORES)	NUMBER OF ANIMALS
I	500	3
II	5,000	3
III	10,000	4
IV	50,000	1

The spore suspension used in this experiment was Vollum-189 strain of B. anthracis which had been stored at 5°C in a concentration of approximately 5×10^{10} spores/ml. This suspension was heat-shocked at 60°C for 30 minutes 48 hours prior to use and standardized to a density of 100,000 spores/ml. All animals were inoculated on the inner surface of the right forearm with appropriate dilutions.

All animals were observed daily for attitude and changes at the site of

* The chimpanzees employed had been used in previous studies on typhoid fever. They were in good health at the time of anthrax challenge.

inoculation and regional lymph nodes. Because of difficulty in handling the animals, other clinical observations were not obtained.

Except for the chimpanzees in the 500-spore group which recovered and were re-challenged, all other animals died. Complete autopsies were performed at death and representative sections were fixed in 10 per cent formalin. Routine histologic procedures were used. All sections were stained with hematoxylin and eosin (H&E). Blood smears were obtained at autopsy.

The three survivors in the 500-spore group were re-inoculated subcutaneously on day 10 with 5000 spores; 25 days after the second challenge the two survivors were inoculated with 50,000 spores. All three died of septicemic anthrax with typical findings at autopsy.

III. RESULTS

A. CLINICAL OBSERVATIONS

The only pertinent observation regularly recorded was that of the evolution of the lesion at the site of inoculation; this process followed essentially the same course as that described in M. mulatta, i.e., there was frequently an edematous, elevated, erythematous lesion developing during the first two days which either regressed in survivors, or became extensive and infiltrative.

Death occurred between three and nine days, often accompanied by extensive cellulitis (which occasionally involved the entire arm) and respiratory distress or other clinical evidence of septicemia, such as weakness, lethargy, depression and coma. Blood smears at autopsy always contained bacilli.

B. GROSS PATHOLOGY

The findings in chimpanzees were essentially similar to those described in M. mulatta, although hemorrhages and hemorrhagic lesions were more frequent.

1. Skin, Site of Inoculation.

As in monkeys, the most conspicuous finding was cellulitis, with infiltrating gelatinous edema, hemorrhage, and involvement of the tributary lymph nodes and neighboring soft tissues.

2. Lymphatic System.

Hemorrhagic and necrotic lymph nodes with or without perilymphatic damage, were essentially similar to those of monkeys. Lymphadenopathy was mostly confined to the tributary lymph nodes, seldom becoming generalized. The spleen was normal in size or enlarged and soft.

3. Respiratory System.

Congestion, disseminated petechiae, and pulmonary hemorrhages were seen in the chimpanzee lungs. Chimpanzees were free of mite lesions with one excep-

tion. Mediastinal edema was not prominent. In two instances marked hydrothorax occurred, one of which was hemorrhagic.

4. Gastrointestinal Tract.

Intestinal lesions caused by the following genera were commonly found: Oesophagostomum, Strongyloides, and, less often, Ascaris. The lesions of oesophagostomiasis were seen in the lower portions of the intestinal tract. For details on these parasites the reader is referred to Ruch's Diseases of Laboratory Primates^{3/}.

5. Central Nervous System.

Only one animal (5000-spore group) had massive hemorrhagic meningitis at autopsy on day 5. Other chimpanzees had either cerebral edema or no significant changes.

6. Cardiovascular System.

In contrast with M. mulatta, the heart was more often damaged by hemorrhages of varying severity, from focal petechial to linear or extensive. In one animal (50,000-spore group) the cardiac hemorrhage was massive and was accompanied by generalized hemorrhage. In two other animals of the 10,000-spore group, generalized hemorrhages were also observed.

7. Other organs.

Focal or extensive hemorrhages were occasionally seen in adrenal glands and ovaries.

C. MICROSCOPIC PATHOLOGY

1. Skin, Site of Inoculation.

Extensive cellulitis, characterized by massive edema, mono- and polymorphonuclear leucocytic infiltration, hemorrhage, and necrosis with myriads of bacilli, was found at the site of inoculation (Figures 1 and 2). This was comparable to that seen in intradermally infected monkeys, although hemorrhage was more prominent and predominantly perivascular, forming multiple, well-circumscribed "candle flame-like" areas. Necrotizing vasculitis, and septic or bacterial thrombi were seen. Widespread cellular infiltrates were especially marked about the blood vessels and necrotic areas. When the lesion was intensely phlegmonous, neighboring striated muscles were involved, appearing as a focal or diffuse myositis.

2. Lymphatic System.

Here as in M. mulatta, the lymphatic tissue response was variable. In the chimpanzee tributary axillary lymph nodes were more damaged than others. In severely affected nodes the principal histologic features were necrosis, hemorrhage, marked depopulation of lymphoid elements, varying numbers of in-

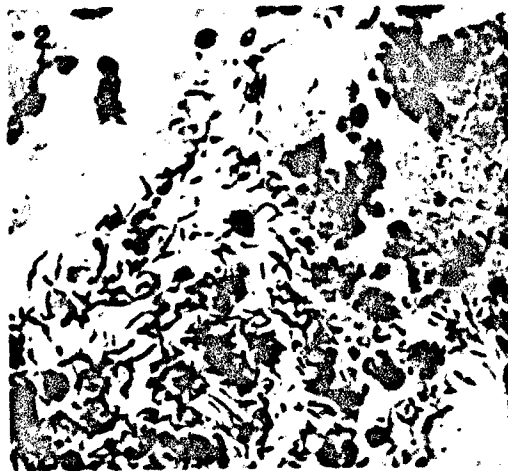
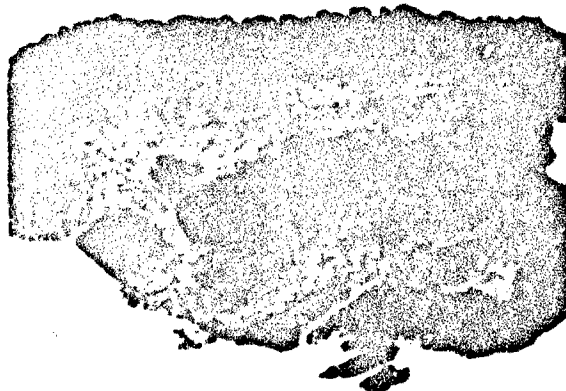


FIGURE 1 SKIN, SITE OF INOCULATION (50,000-SPORE GROUP, DAY 4) CELLULITIS, EXTENSIVE EDEMA & NECROSIS WITH WELL CIRCUMSCRIBED, "FLAME-LIKE" PERIVASCULAR HEMORRHAGE & INTACT EPITHELIAL SURFACE. H & E. X6. ACC. 1298

FIGURE 2 SKIN, SITE OF INOCULATION (5,000-SPORE GROUP, DAY 5) CELLULITIS, PERIVASCULAR HEMORRHAGE, FRAMED BY MASSIVE INVASION OF BACILLI (NECROSIS), EDEMA & INFLAMMATORY REACTION H & E X432 ACC. 1243

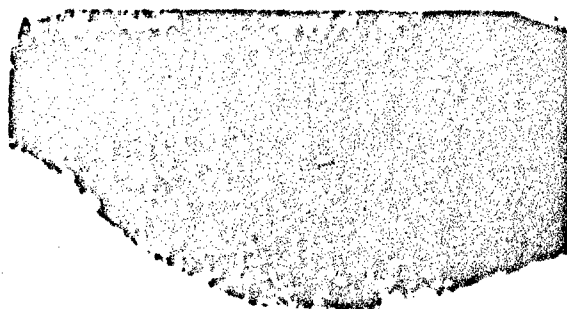


FIGURE 3 LYMPH NODE, AXILLARY, TRIBUTARY OF THE SITE OF INOCULATION (50,000-SPORE GROUP, DAY 4). MASSIVE HEMORRHAGE WITH ALMOST COMPLETE DESTRUCTION. ONLY A FEW FRAGMENTS OF LYMPHOID FOLLICLES ARE VISIBLE AT THE PERIPHERY & AT THE CENTRAL PORTION. H & E (A) X6 (B) X51. ACC 1298.

flammatory cells, and masses of bacilli (Figure 3). Hemorrhage was frequently predominant, in contrast with findings in the monkey. Necrotizing vasculitis was occasionally encountered in the nodes.

The degree of splenic involvement also varied intensively from one animal to another. Hemorrhage and necrosis (Figure 4) occurred frequently, accompanied by an intense cellular infiltrate and myriads of bacilli. All degrees of damage were observed.

3. Respiratory System.

Lesions of pulmonary acariasis were seen in only one chimpanzee. Occasionally localized fibrosis with or without inflammatory reaction and concomitant atelectasis were found with no parasites visible. These were attributed to other causes, possibly other parasitic infestations.

The pulmonary changes were comparable to those seen in the monkey. However, pulmonary hemorrhages and hemorrhagic pneumonitis were more extensive, although severity was variable. In the chimpanzee, pulmonary hemorrhage was accompanied by severe congestion and focal edema (Figure 5). Diffuse, generalized pulmonary edema was occasionally observed.

Numerous bacilli were frequently present within the alveolar capillaries. Multiple clumps or large aggregates of bacilli were often observed throughout the lung parenchyma, especially when severe hemorrhagic pneumonitis was present (Figure 4). Often this bacillary invasion was associated with bacterial thrombi.

4. Cardiovascular System.

Focal and generalized hemorrhages were frequently observed and were probably related to the vascular damage, obvious capillary rupture, and possibly damage of larger vessels. The necrotizing vasculitis, intimately related to septicemia, and found in several organs and frequent accumulations of bacilli around blood vessels support this observation.

Hemorrhage and necrosis involving the endocardium, myocardium and epicardium were seen more frequently in chimpanzee heart than monkey. Although extensive pericardial and subendocardial hemorrhages associated occasionally with myocardial necrosis were observed, there was no evidence of substantial inflammatory reaction (Figure 6). Collections and masses of bacilli were found either about the blood vessels or in the damaged areas.

5. Gastrointestinal Tract.

Hemorrhages, erosion, and ulceration were frequently observed in the intestinal mucosa associated with parasitic lesions. Cysts or nodules containing one or more nematodes were seen in the submucosa or mesenteric attachments of the lower colon. Frequently sections of nematodes or their larval forms were seen within the intestinal mucosa. Many of these parasitic lesions contained bacilli (Figure 7). Eventually, these superinfected lesions appeared as necrotizing hemorrhagic, ulcerative enteritis densely populated with bacilli.



FIGURE 4. SPLEEN. (10,000-SPORE GROUP, DAY 4). H & E.
(A) ACC. 734 DESTRUCTION ALMOST COMPLETE BY HEMORRHAGE
& NECROSIS. X 250.
(B) ACC. 622 LESS DESTRUCTION. X 57.

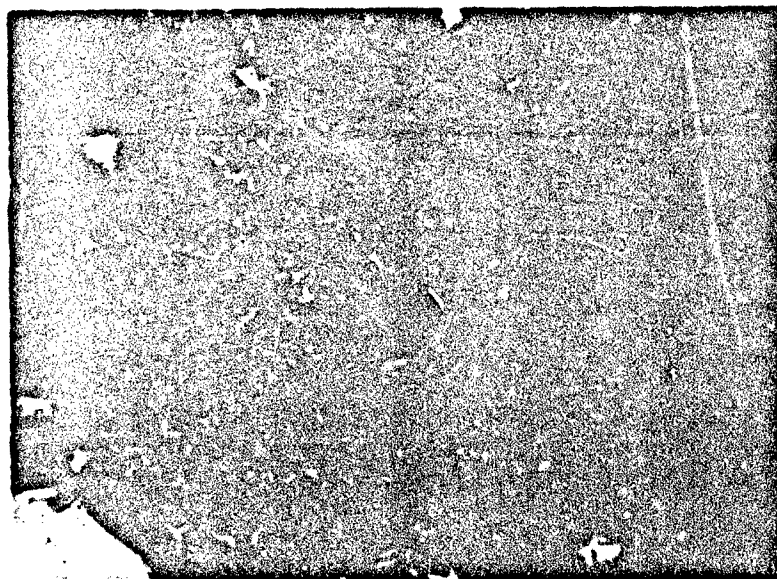
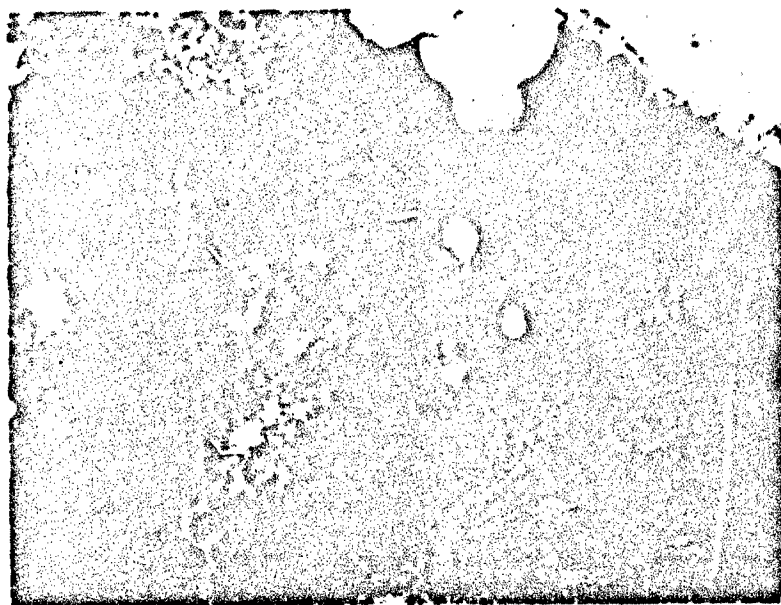


FIGURE 5. LUNG. (10,000-SPORE GROUP, DAY 4) HEMORRHAGIC PNEUMONITIS. H&E. ACC. 734
(A) EXTENSIVE PULMONARY HEMORRHAGE ASSOCIATED WITH EDEMA,
MODERATE INFLAMMATORY INFILTRATE. X 51.
(B) MASSES OF BACILLI. X 432.

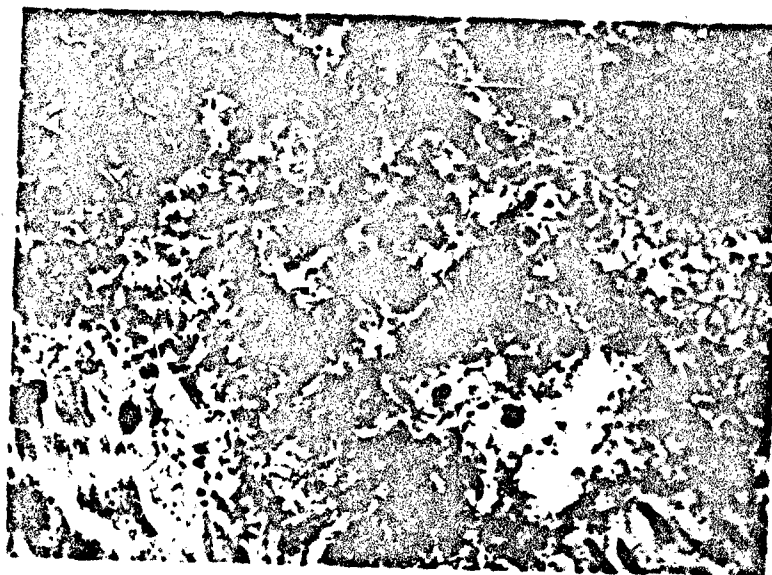


FIGURE 6. HEART. (10,000-SPORE GROUP, DAY 4) SUBENDOCARDIAL HEMORRHAGE & NECROTIZING MYOCARDITIS. H & E. ACC 622
 (A) LEFT VENTRICLE WITH ENDOCARDIUM ABOVE & A SMALL PORTION OF RELATIVELY INTACT MYOCARDIUM IN THE RIGHT LOWER CORNER. EXTENSIVE & MASSIVE SUBENDOCARDIAL HEMORRHAGE WITH MASSES OF BACILLI (IN BLACK) SWEEPING THE SURFACE. X49.
 (B) NECROTIZING MYOCARDITIS WITH INTENSE BACILLARY INVASION. X432.

6. Liver.

No extensive necrosis was observed. The findings can be summarized as: varying degrees of congestion and edema associated with minimal to moderate hepatocellular degeneration and small foci of necrosis, always accompanied by many bacilli (Figure 8).

7. Endocrine Glands.

Focal or diffuse hemorrhages of adrenal cortex, generally accompanied by necrosis and partial destruction of the parenchyma, were found in one or both glands (Figure 9). Large numbers of bacilli were associated with these damaged areas. The medulla was never involved.

In two of seven females, the ovaries were the site of marked hemorrhagic oophoritis with myriads of bacilli and parenchymatous destruction.

Other glands were consistently spared.

8. Central Nervous System.

Massive hemorrhagic meningitis, with extensive inflammatory reaction (monocytes and polymorphonuclear cells), diffuse necrosis and massive invasion of bacilli was seen in one 5000-spore animal (Figure 10).

9. Genitourinary System.

Hemorrhages were frequently observed in the organs of this system. However, no extensive lesions or substantial tissue destruction were seen.

Renal tubules in two animals contained an eosinophilic, fine, granular, protein-like precipitate with little or no damage to the epithelial lining. No blood or hemoglobin casts were recognized in the tubules to warrant a diagnosis of "lower nephron nephrosis" or "acute tubular necrosis." In all cases the glomeruli contained varying numbers of bacilli, and small bacterial thrombi were occasionally found within the renal parenchyma.

An acute, hemorrhagic, ulcerative pyelitis with concomitant cystitis, containing bacilli was seen in one animal. It was not clear whether a pre-existing lesion was present and was infected with anthrax or whether the lesion was the result of septicemia. Figure 11 illustrates this ulcerative lesion with collections of bacilli.

10. Musculoskeletal System.

Apart from the myositis described at the site of inoculation, no significant pathologic changes were observed in muscle. Small foci of necrosis generally containing bacilli, were often encountered in the bone marrow.

11. Thymus.

As in monkey, the thymus was not affected.

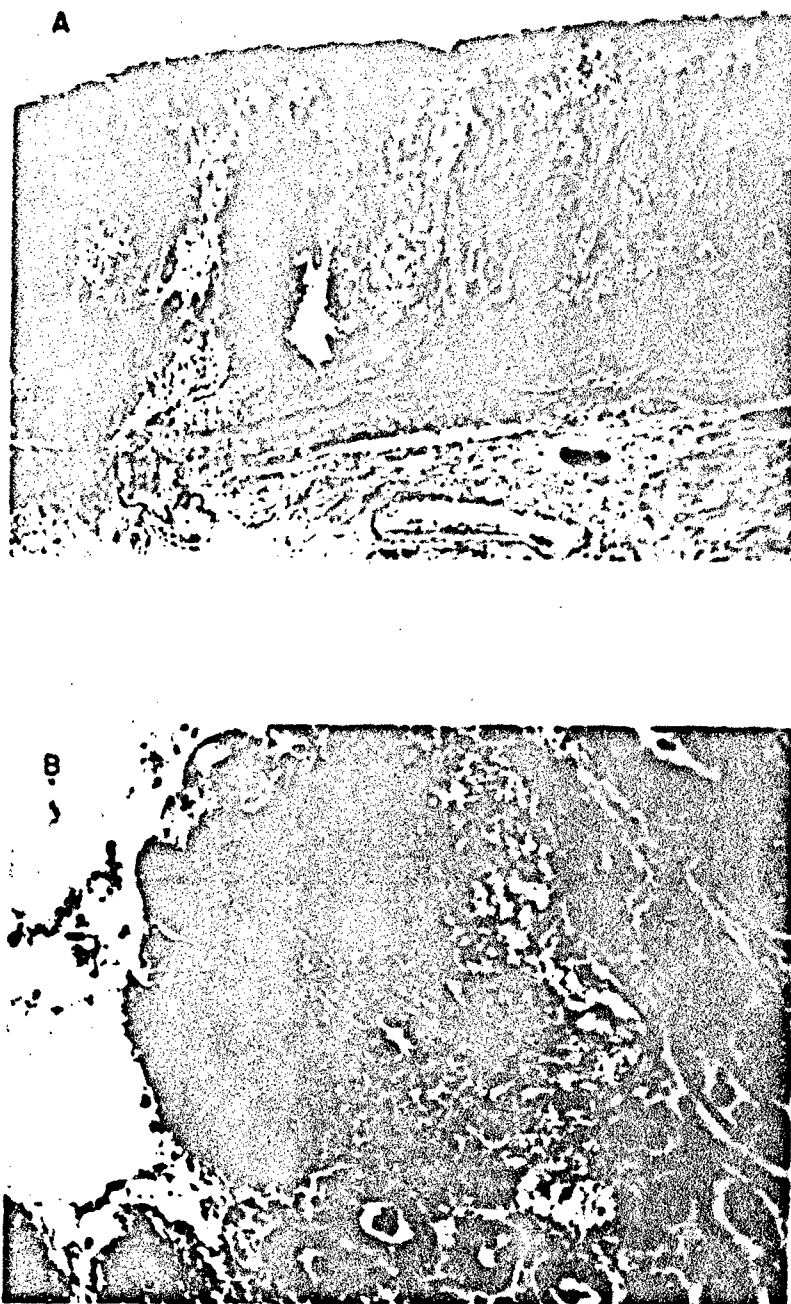


FIGURE 7. GASTROINTESTINAL TRACT. (50,000-SPORE GROUP, DAY 4)
SUPERIMPOSITION OF ANTHRAX ON A PRE-EXISTING PARASITIC
LESION. H & E. ACC. 1298.
(A) NECROTIZING, HEMORRHAGIC, ULCERATIVE ENTERITIS WITH
MASSES OF BACILLI. X51.
(B) ACCUMULATION OF BACILLI ON THE SURFACE EPITHELIUM. X197.

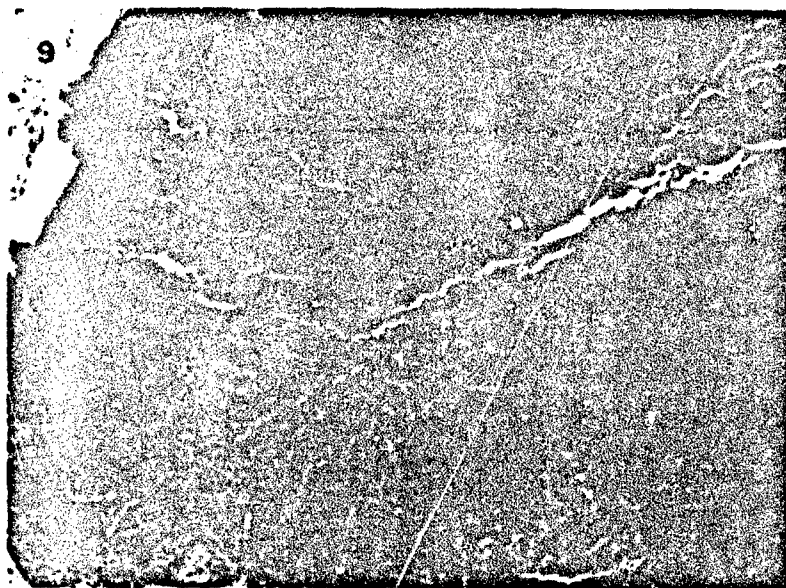


FIGURE 8 LIVER (10,000-SPORE GROUP, DAY 4). FOCAL NECROSIS WITH NUMEROUS BACILLI. H & E X175. ACC. 622.
FIGURE 9 ADRENAL (10,000 SPORE GROUP, DAY 7). EXTENSIVE CORTICAL HEMORRHAGE & NECROSIS. CORTICAL STRUCTURE CAN STILL BE FOLLOWED IN THE LOWER PORTION. H & E. X51. ACC. 735.



10



11

FIGURE 10 MASSIVE MENINGITIS (5,000-SPORE GROUP, DAY 5) MASSIVE
BACILLARY INVASION, NECROSIS, & NUMEROUS INFLAMMATORY CELLS
MONONUCLEAR & POLYMORPHONUCLEAR. H & E X432. ACC 1243

FIGURE 11 KIDNEY PELVIS (50,000 SPORE GROUP, DAY 4) NECROTIZING,
HEMORRHAGIC ULCERATIVE PYELITIS WITH MASSIVE INVASION OF
BACILLI H & E X49

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IV. DISCUSSION

A comparison of these animals dying at different times, revealed that the principal lesions were hemorrhage and necrosis. The hemorrhagic lesions were focal, multiple, or generalized; their frequency did not appear to be dose-dependent. It is assumed that the hemorrhages were intimately related to septicemia.

Except for the 500-spore group which survived, all the chimpanzees died between 3 and 7 days post-primary challenge. This is in contrast with monkeys which succumbed even at the 500-spore level. No significant difference was found in the times of death between the original groups (Table II). There is a suggestion in the death times that the Group I animals were somewhat more resistant when reinoculated with higher doses of spores.

TABLE II. SURVIVAL TIME OF CHIMPANZEES INFECTED SUBCUTANEOUSLY WITH B. ANTHRACIS

GROUP	DOSE (Spores)	NO. DEAD/TOTAL	TIME OF DEATH (days)	
			Range	Avg
I	500	0/3	-	-
II	5,000	3/3	4-5	4.6
III	10,000	4/4	3-7	4.5
IV	50,000	1/1	4	4
Re-challenge of Group I survivors				
	5,000	1/3	9	9
	50,000	2/2	5-6	5.5

The principal lesions as listed previously in intradermal anthrax in the monkey¹, were also observed in chimpanzees with a few minor differences. Except for the tributary lymph nodes, the lymphatic system was less affected than that of the monkeys. The lymph nodes and spleen were more hemorrhagic than necrotic. Extensive necrotic lysis usually seen in monkeys was replaced by focal or patchy necrosis and more prominent hemorrhage.

Even though the respiratory system in this host was not affected by mite lesions, the lungs were the site of frequent hemorrhages, hemorrhagic pneumonia, intense congestion associated with edema, and invariably myriads of bacilli with concomitant bacterial thrombi. This finding is comparable to that of Albrink and Goodlow² in chimpanzees infected with anthrax by the respiratory route.

Frequent cardiovascular hemorrhagic changes observed in these animals were contrasted with occasional such local lesions in monkeys.

In contrast with the monkey, ulcerative hemorrhagic enteritis with myriads of bacilli was a constant finding.

It is noteworthy that in both species dying from septicemia and its complications, the nature and distribution of lesions, and the relative sensitivity of organs remain essentially unchanged, with only two exceptions. The exceptions are the cardiovascular system and the lungs in which in the chimpanzee there were more severe and extensive hemorrhages.

These studies indicate that, as in the monkey, after the development of the local lesion at the site of inoculation, the blood and the lymphatic system are concurrently the first targets for B. anthracis spores. The dissemination of organisms and generalization of anthrax infection are subsequently achieved by these two systems; here as in the monkey, there is no clear-cut explanation as to which route prevails. However, the presence of more hemorrhagic lesions and multiple visceral hemorrhages seem to indicate a blood-borne dissemination. The constant presence of bacilli in the glomerular tufts and frequent bacterial thrombi in a relatively resistant organ such as kidney with no significant pathologic changes also favor the blood route predominance. Furthermore, severe damage to the axillary lymph nodes (tributary to the site of inoculation) with relative sparing of other nodes favors this route.

V. SUMMARY

A comparison was made of the pathogenesis and histopathologic evolution of experimental anthrax in the chimpanzee and monkey (M. mulatta).

Although the lesions were essentially similar and due to septicemia in both hosts, anthrax infection engendered more hemorrhage and hemorrhagic lesions in the chimpanzee.

Superinfection of anthrax on a pre-existing intestinal parasitic lesion was a rule, rather than an occasional finding, as in the monkey.

Chimpanzees survived a low-dose challenge of 500 spores, but succumbed to re-challenges with higher doses. Monkeys died at such a challenge level.

Germination of B. anthracis and initiation of lesions at the site of inoculation and the generalization of the infection by blood or lymphatic systems were discussed. The evolution appears to be similar in both hosts.

Despite differences in routes of infection, these chimpanzees responded in a comparable manner to chimpanzees infected by the respiratory route as described by Albrink².

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STUDIES ON BACILLUS ANTHRACIS

PART 7

OBSERVATIONS ON PENICILLIN PROPHYLAXIS OF
EXPERIMENTAL INHALATION ANTHRAX IN THE MONKEY
(Gochenour, Gleiser, and Tigertt)I. INTRODUCTION

Anthrax in the Rhesus monkey (Macaca mulatta) induced by respiratory exposure to spores of a virulent strain of Bacillus anthracis in doses greater than 1×10^5 spores is a rapidly fatal illness, death occurring by the sixth day. The clinical manifestations are undramatic and inconsistent; Fever, respiratory distress, depression, or convulsions prior to exitus may or may not be manifest. At autopsy, massive bacteremia is characteristic. Common gross findings are intrathoracic lymphadenopathy, lung hemorrhages, edematous mediastinitis, adrenal hemorrhage, splenomegaly, hemorrhagic meningitis, and hydrothorax^{1/}.

In a series of experiments on prophylaxis of inhalation anthrax in the monkey, Henderson et al^{2/} found that a 5-day course of intramuscular (IM) procaine penicillin, 150,000 units daily, initiated 24 hours post-exposure, merely delayed the times to death of animals so treated when compared to those of non-treated controls. When, however, they supplemented the same regimen with two doses of protective antigen^{3/}, the first at 24 hours post-exposure, and the second 10 days later, all animals survived respiratory infection fatal to control animals.

In the course of studies conducted by the authors and their associates^{4/} on respiratory anthrax in sheep, prophylaxis was initiated 24 hours post-exposure. Five therapeutic regimens were employed. Protective antigen alone had no effect on the infection. All animals in each of the four other groups survived. No evidence of a requirement for administration of protective antigen for successful prophylaxis of respiratory anthrax in the sheep was afforded by this experiment.

The requirement for administration of protective antigen to the monkey for successful prophylaxis of inhalation anthrax and the lack of such requirement for successful prophylaxis in the sheep may be reconciled by either of two hypotheses, both compatible with the observed essentially similar pathogenesis of respiratory anthrax in these two species.

First, unlike the sheep, the monkey may be unable to respond adequately, if at all, to anthrax antigens elaborated during his infection prior to antibiotics. This might be attributable to failure to recognize and respond to protective antigen in its combined native state, or to a slow rate of immune response to the antigenic stimulus.

Second, the rate of entrance into the lymphatics by the spores and their subsequent germination and invasion of the blood stream in the monkey may be

markedly less rapid than in the sheep. Early attainment of bactericidal blood levels of penicillin would under these circumstances destroy the bacilli as rapidly as they entered the blood stream, thus depriving the monkey of any significant antigenic stimulus to antibody production. The sheep, on the other hand, might well have had experience with relatively large numbers of blood-borne bacilli and thus have received an adequate antigenic stimulus prior to the attainment of bactericidal blood levels of penicillin. This would result in monkeys vulnerable to infection with anthrax after cessation of antibiotic administration and sheep "in a continuous state of prophylactic readiness."^{2/}

If the first hypothesis is correct, prophylaxis alone should be unsuccessful regardless of the time of its initiation. If the second were correct, prophylaxis with penicillin alone should be unsuccessful if initiated early post-challenge and successful if delayed.

The studies reported herein were conducted to test these hypotheses. An effort was made to replicate in so far as possible the conditions under which the experiments of Henderson and his associates^{2/} were conducted.

II. MATERIALS AND METHODS

Vollum-189 strain of B. anthracis was employed. It was prepared in 1957 and was stored as a phenolated spore suspension (4×10^{10} /ml) at 5°C until used. Spore suspensions were diluted in distilled water and heat-shocked at 60°C for 30 minutes prior to aerosolization. The guinea pig subcutaneous LD₅₀ of this suspension has remained constant at <4 spores.

The device used for respiratory exposure generated a dynamic aerosol cloud at the rate of 20 standard cubic feet per minute; humidity was controlled at 80 percent for all exposures. A Collison generator^{3/} was used to disseminate the spores. This fixture produces a predominantly small particle aerosol with a mass median diameter of approximately 1.5 μ . The monkeys were exposed in helmets through which the aerosol flowed at a rate of 25 L/min, and were calculated to have breathed approximately 1 L/min. Estimation of aerosol concentration presented to the monkeys was made by examination of samples obtained from all glass impingers (AGI-30's) in the effluent air lines from the exposure helmets.

Twenty M. mulatta, ranging in weight from 1.6 to 3.1 kg were used. The drug regimen employed was five single daily doses of 150,000 units procaine penicillin IM for a total of 750,000 units. Drug was started at 24, 48 and 72 hours. Six untreated animals served as controls. Blood cultures were obtained on all animals at the time of initiation of therapy. The control animals were examined for bacteremia on days 1, 2 and 3.

III. RESULTS

Respiratory doses presented to the monkeys ranged from 345,000 to 1,200,000 spores with a geometric mean of 783,000.

The six non-treated control animals succumbed to the infection, deaths

occurring on days 2, 3 and 4. Five of the six had demonstrable bacteremia prior to death. All were grossly bacteremic at death. Fever was noted in only two animals.

Four of five monkeys placed on penicillin prophylaxis at 24 hours succumbed. Deaths occurred on the fourth (2 animals), eighth and ninth days after cessation of therapy. None of the animals had demonstrable bacteremia or fever at time of initiation of penicillin. No bacteremia was demonstrable prior to death in one animal grossly bacteremic at death on the fourth day after cessation of penicillin. Fever and bacteremia were present from two to three days prior to death in the three others that died. The remaining animal survived, despite fever and bacteremia on the tenth through thirteenth post-prophylactic days.

One of five monkeys, 48 hour drug group, died on the fifth day after cessation of drug. This animal was bacteremic at the time of initiation of penicillin. It experienced two days of fever prior to death, at which time B. anthracis was isolated from the blood. The four remaining animals in this group survived. Three were bacillemic at the time prophylaxis was started, at which time one was febrile. This was the only animal in the group which remained afebrile after cessation of penicillin. The single animal with a negative blood culture at the time penicillin was started, was febrile on the eleventh through fourteenth days after drug was discontinued. Bacteremia was demonstrated on the last day of the febrile episode.

Two of the four monkeys in which prophylaxis was delayed until 72 hours post-exposure succumbed to the infection while on therapy. Both were bacteremic at the time of initial administration of the drug and one had been febrile for two days. The other two animals were both febrile and had negative blood cultures at the time they received their first dose of penicillin. Both were afebrile thereafter; bacillemia was not demonstrable.

Table I summarizes the significant gross autopsy findings on the animals succumbing.

All surviving monkeys were observed for a period of 31 days after exposure at which time they were inoculated subcutaneously with 5,000 heat-shocked spores. The animals at this time had serologically demonstrable antibody against B. anthracis. All survived without ill effect.

IV. DISCUSSION

The results obtained indicate that the monkey, like the sheep, is capable of responding to in vivo elaborated anthrax antigens. Under circumstances in which an adequate antigenic stimulus is presented to the monkey, its response is sufficiently rapid to attain, within the period of antibiotic cover, a level of immunity sufficient to permit the monkey to cope with the remaining anthrax organisms as they leave the lungs and gain entrance to the body.

It is apparent that the time of initiation of bactericidal drug is critical. As in the experiments of Henderson et al², the monkeys treated at 24

TABLE I. PRINCIPAL GROSS AUTOPSY FINDINGS ON MONKEYS SUFFERING TO INHALATION ANTRAX

PRINCIPAL GROSS LESIONS	PENICILLIN-TREATED MONKEYS									
	NON-TREATED MONKEYS									
	943	947	949	916	938	912	Death during Ex ^a /		Death after Ex ^b /	
							950	940	7-19	918 924 946 751
Intrathoracic										
Lymphadenopathy	-	+	+	+	+	+	+	+	+	+
Lymph node hemorrhage	-	-	-	-	+	+	-	-	-	+
Mediastinal										
Edema	-	-	-	+	+	+	-	-	-	+
Hemorrhage	-	-	-	-	-	-	-	-	-	+
Hydrothorax	-	-	-	-	-	+	-	-	-	+
Pulmonary										
Parenchymal hemorrhage	+	-	+	+	-	+	+	-	-	-
Hemorrhagic nodules	-	-	-	-	+	-	-	-	-	+
Meningeal hemorrhage	-	-	-	-	+	+	+	+	-	+
Splenomegaly	+	+	-	-	+	-	-	-	+	-
Day of death (post-exposure)	3	2	4	2	4	4	4	4	13	11 9 14 9
Febrile illness	-	-	-	-	+	+	-	+	+	-

^a/Therapy initiated 72 hours post-exposure.^b/Therapy initiated 24 hours post-exposure except Monkey No. 918, 48 hours post-exposure.

hours were essentially without benefit of antigenic stimulus and remained vulnerable to infection. Delay until 72 hours on the other hand permitted this rapidly fulminating infection to pass beyond the point of no return in half the animals so treated.

It is of note that the surviving monkeys did not universally attain complete refractoriness to infection after drug cessation. It is strongly suggested by the post-therapy fevers in four, and demonstrable bacteremia in two monkeys, suggest that the response in some may be barely sufficient to swing the balance in the favor of the animal.

These experiments further suggest that had penicillin prophylaxis been initiated earlier in the above-mentioned studies⁴ in sheep, a requirement for the administration of protective antigen for successful prophylaxis might have been demonstrated.

Intrathoracic lymph node and mediastinal involvement were much more extensive and severe in the animals dying after cessation of early penicillin prophylaxis than in non-treated animals. This, coupled with the higher incidence of fever in the treated group, suggests that the disease in such animals more nearly simulates human respiratory anthrax. Rhesus monkeys may serve as appropriate models for study of early diagnosis and therapy.

V. SUMMARY

The result of penicillin prophylaxis of experimental inhalation anthrax in the Rhesus monkey has been shown to be dependent upon the time of its initiation. If begun too early, it is unsuccessful. It may not be too long delayed, or the infection will have passed the point of no return. A brief, critical time period exists, during which successful prophylaxis may be initiated. This favorable outcome is attributed to the elaboration in vivo of sufficient antigen (s) to stimulate an adequate immune response, prior to the initiation of antipiosis.

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